THE JOURNAL OF MEDICAL RESEARCH

CORRECTION. — PLATE IX. — Fig. 2 *should read* Fig. 3.
Fig. 3 *should read* Fig. 4.
Fig. 4 *should read* Fig. 2.

VOLUME VII.
(New Series, Vol. II.)

JANUARY TO MAY, 1902

BOSTON
MASSACHUSETTS
U.S.A.

THE JOURNAL OF MEDICAL RESEARCH

Continuation of the

JOURNAL OF THE BOSTON SOCIETY OF MEDICAL SCIENCES

EDITED BY

HAROLD C. ERNST, M.D.

VOLUME VII.

(New Series, Vol. II.)

JANUARY TO MAY, 1902

BOSTON
MASSACHUSETTS
U.S.A.

INDEX TO SUBJECTS.

PAGE

M.

P.

R.

S.

T.

V.

INDEX TO NAMES.

THE

Journal of Medical Research.

(New Series, Vol. II.)

Vol. VII. JANUARY, 1902. No. 1.

NOTES ON THE OCCURRENCE AND HABITAT OF ANOPHELES PUNCTIPENNIS AND ANOPHELES MACULIPENNIS IN THE VALLEY OF THE ANDROSCOGGIN.

Edwin O. Jordan.
(*University of Chicago.*)

The attempt to prevent malarial infection by combating the particular kind of mosquito that forms the intermediate host of the malarial parasite is not likely to meet with even moderate success unless fortified by a complete knowledge of the development and ecology of the various members of the incriminated genus. It is believed by many that one of the most hopeful measures for diminishing malaria lies in the discovery and reproduction of the natural conditions hostile to Anopheles. As pointed out by several writers, such conditions assuredly exist, or Anopheles would be far more generally distributed than is actually the case. What these conditions are can be determined only through systematic study of the local conditions in different regions by a number of different observers. The following notes of one summer's collections are recorded in the hope that they may be found useful in further comparative studies.

In many respects the history and distribution of malaria in New England offers a particularly inviting field for investigation. Malaria was not one of the diseases with which the early settlers had seriously to contend; according to Dr. Holmes,[1] the records of the first century of New England indicate that "indigenous intermittent fever can have pre-

[1] O. W. Holmes. Facts and Traditions respecting the Existence of Indigenous Intermittent Fever in New England. Boylston Prize Essay, 1836.

vailed but to a very limited extent, and the only place which we can clearly point to as giving origin to the disease is New Haven." Adams,[1] writing in 1880, states that "During the present century it has been, with very trifling exceptions, unknown as an indigenous disease. . . . But in 1850 this disease obtained a foothold in Connecticut, on the shore of Long Island Sound, and about 1864 began a northward march across the western half of Connecticut, and has steadily pushed forward, until, in 1876, it reached the northern border of the State, and thence, ignoring the boundary line, stepped over into Massachusetts, appearing in Sheffield in 1877. But, previous to this date, a few cases had appeared in three localities; viz., in Springfield since 1870, New Marlborough since 1874, and Holyoke since 1875. In 1878 the disease appeared at Agawam and New Lenox; and in 1879 and 1880 it made a rapid advance, invading a considerable number of towns."

Since 1880 malaria of the tertian type has prevailed quite extensively in many parts of New England and has invaded many districts previously exempt. Many of the surburban towns around Boston have suffered rather severely during the last decade. Prior to this time indigenous malaria, save a few scattered cases which attracted attention from their rarity,[2] had been practically unknown in Boston and its environs. The towns of Central and Western Massachusetts, especially those along the valley of the Connecticut and among the Berkshire Hills, appear to have harbored some

[1] J. F. Alleyne Adams. Intermittent Fever in Massachusetts. Second Annual Report of the Massachusetts State Board of Health, Lunacy, and Charity. Supplement, Public Health, 1880.

[2] An interesting Report on Intermittent Fever in Chelsea, by Drs. H. I. Bowditch, John Ware, and Ephraim Bush, in the Boston Medical and Surgical Journal, Vol. 47, p. 535, Jan. 26, 1853, records several cases of fever and ague originating near the edge of a "partially dried-up marsh." In this report the statement is made that data extracted from a diary kept by the Rev. Noadiah Russell in 1682–3, while a tutor at Harvard, indicate that at that date fever and ague were prevalent in the neighborhood; the diarist himself had an attack in 1682. Dr. Holmes (op. cit.) states that Urian Oakes, President of Harvard College from 1675 to 1681, suffered with a quartan ague perhaps contracted in this country. Five cases of intermittent fever originating in Boston between 1865 and 1868 are recorded by Treadwell (Boston Med. and Surg. Journal, 1868, Vol. 78, p. 227).

malaria in early times and to have suffered up to the last decade much more than the eastern portion of the State.

In Connecticut and Rhode Island it seems that malaria always prevailed more extensively than in Massachusetts. Dr. Holmes (op. cit.) refers to New Haven as the only locality in New England where the evidence of the existence of indigenous malaria in colonial times is incontrovertible. Adams (op. cit.) gives a list of thirty-five towns and villages in Connecticut in which malaria appeared between 1850 and 1878; and Chapin[1] gives a long list of Rhode Island towns in which malaria was known to occur in 1881; a "large number of cases" were reported as occurring in Providence in that year.

The three northern New England States appear to have been almost entirely exempt through their whole history. There is no good evidence that indigenous malaria has ever existed in Maine; one case occurring at Poland is mentioned by Dr. Holmes, but this is rejected by Adams on diagnostic grounds; one case occurring at Biddeford about 1760 was indigenous "according to tradition." Upon the map accompanying Dr. Holmes's paper, Burlington in Vermont, and Kensington in New Hampshire, are the sole localities in these States which are designated as places where intermittent fever is "supposed to have originated." Adams (op. cit.) says: "It is not known that intermittent fever has appeared farther north than Massachusetts." Chapin (op. cit.) reports a few cases occurring at Keene, New Hampshire, in 1883, and notes that some other cases have occurred in towns along the valley of the Connecticut. On the whole, however, it is clear that the disease has been far less prevalent in the northern than in the southern portion of New England.

Some interesting questions are raised by the historical record. It may be asked, for instance, whether the comparative exemption of the early settlers was due to the absence of Anopheles either wholly in some localities or in sufficient

[1] Chapin C. V. The Origin and Progress of the Malarial Fever now prevalent in New England. Fiske Fund Prize Dissertation, No. XXXII., 1884.

numbers in others, or whether it was due chiefly to the scarcity of individuals harboring the malarial parasite from whom the Anopheles might become infected. It may be asked also what was the cause of the malarial invasion of New England which began in the southern portion about the year 1850, and has progressively extended its range northward, until at the present time many localities, previously free throughout their history, such as the lower valley of the Charles River, are thoroughly impregnated with the germs of the disease? Why, again, have the more northern New England States escaped malaria almost wholly, although no more sparsely settled than other regions in which malaria has occurred? If, as seems probable, the full answers to the questions raised above are connected with the presence or absence of special topographical or seasonal conditions inimical to special kinds of mosquitoes, the importance of setting on foot coöperative investigations into the ecology of Anopheles cannot be mistaken.[1]

New England would seem to be an especially favorable field for such a study. Making all due allowance for the inadequacy of the historical record, it is nevertheless true that this portion of the United States contains a relatively large proportion of municipalities possessed of fairly precise information regarding their early medical history. A reëxamination of the available historical data might throw light on some of the obscurer points at issue. The recent extension of the disease, leading to the infection of communities which had hitherto enjoyed immunity, offers a definite and promising field for inquiry. The occurrence and especially the relative seasonal abundance of Anopheles in various localities is one of the more important points to be determined, and it would seem as if coöperative investigation along this line should be especially fruitful.

The locality in which my own observations have been made during the summer of 1901 is in the neighborhood of

[1] The employment of bands of Italian workmen for the construction of water-works and sewerage systems, whatever significance it may have for particular localities, can only partially explain the range and spread of the disease.

the town of Shelburne, N.H., in the valley of the Androscoggin River, and near the Maine border. The town is sparsely settled, and consists of scattered farm-houses strung along a strip of cultivable meadow-land barely thirteen hundred feet wide; the river bottom is here about seven to eight hundred feet above sea level. From the intervale, hills rise, sometimes quite abruptly, to a height of twenty-five to thirty-five hundred feet. The mean temperature in the summer is, as a rule, under 70° F. (cf. Hitchcock, "Geology of New Hampshire," p. 144. June, 62.9°; July 69.3°; August, 64.1°; September, 55.4°). The region is considered a healthy one, and so far as I can discover from the testimony of local physicians and others, no cases of malaria have ever originated in Shelburne or its vicinity.

My collections have extended over the period from June 21st to September 21st. Two related lines of work have been pursued: the collection of the winged insects, and the collection of the larvæ and study of the larval habitat.

RELATIVE ABUNDANCE OF ADULT ANOPHELES.

Very few winged insects belonging to the genus Anopheles were found during the entire season. I never observed adult Anopheles in the immediate neighborhood of their breeding-places, although I carefully scrutinized every mosquito that approached, and captured and examined hundreds of other mosquitoes in the course of the summer. The house occupied by the writer was the object of special search, since, as is well known, most species of Anopheles are semi-domestic, and tend to congregate in dwellings and their immediate neighborhood.

From the beginning of the season daily search was instituted for the mosquitoes that had found their way into the house, which, although quite carefully screened, was not mosquito-proof. The following table gives the results of the findings:

	A. punctipennis.	C. triseriatus.	C. stimulans.
June 27	0	2	0
June 28	0	1	0
June 29	0	2	0
July 2	0	4	0
July 4	0	2	0
July 5	0	2	0
July 11	0	1	0
July 13	1	0	0
Aug. 8	0	1	0
Aug. 12	1	0	0
Aug. 15	0	0	1
Aug. 17	0	1	0
Aug. 25	0	0	1

A similar but less systematic hunt was made for mosquitoes on the porch of the house and on the outside of the screens in the evening, with the following results:

	A. punctipennis.	C. triseriatus.	C. stimulans.	C. pungens.
June 21 . . .	1[1]	15	0	0
June 22 . . .	1[2]	14	0	0
June 24 . . .	0	2	0	0
June 25 . . .	0	5	1	0
June 26 . . .	0	4	1	0
June 28 . . .	0	1	0	0
July 1 . . .	0	0	1	0
July 14 . . .	0	1	1	0
Aug. 5 . . .	1[3]	0	0	0
Aug. 21 . . .	0	0	4	0
Aug. 25 . . .	1[2]	0	0	1
Aug. 27 . . .	0	0	1	0
Sept. 4 . . .	0	0	2	0
Sept. 5 . . .	0	0	1	0
Sept. 7 . . .	0	0	0	0

1 Killed in act of biting, on porch of house, 10 A.M.
2 Caught on outside of screen with other mosquitoes in evening.
3 Killed in act of biting, 7.30 P.M.

In the course of the whole season, therefore, only six specimens of A. punctipennis were captured in and about the house as against seventy-four specimens of Culex. The actual disproportion between the two genera is somewhat greater than these figures indicate, since many mosquitoes which were killed by a blow of the hand were injured too seriously for identification of the species and were not included in the table. It was, however, possible in these cases to distinguish readily between the genera Culex and Anopheles, and all the individuals so examined proved to be Culex with the two exceptions (June 21, August 5) above noted. Some fifty-three individuals belonging to unidentified species of Culex should be added to the total number of this genus. In this locality, therefore, during the season of 1901, the proportion of Anopheles found in the house and in its immediate neighborhood was about 4.5 per cent. of the mosquito fauna (6 Anopheles punctipennis, 58 C. triseriatus, 14 C. stimulans, 2 C. pungens, 53 C. sp?).

If the mosquitoes that were encountered along the roadside or in the woods were to enter into consideration, the proportion of mosquitoes belonging to the genus Anopheles would be lower still, since not a single Anopheles imago was captured away from the neighborhood of the house, whereas hundreds of mosquitoes belonging to the other genus were caught with the cyanide bottle or otherwise killed.

There are no definite statements regarding the relative abundance of the two genera in other parts of New England. The impression obtained from the Notes published by Theobald Smith[1] is that the proportion of Anopheles was somewhat greater in the neighborhood of Boston in 1900 than I have found it in Shelburne in 1901, and a few scattering observations made in the Boston suburbs (Newton, Mass.) by the writer in the early summer of 1900 tend to strengthen this impression. There is also very little to be found in the general literature of the subject

[1] Theobald Smith. Notes on the Occurrence of *Anopheles punctipennis* and *A. quadrimaculatus* in the Boston Suburbs. Journal of the Boston Society of Medical Sciences, 1901, V., p. 321.

regarding the numbers of Anopheles present in various localities, even in the more carefully studied malarious districts. (Cf. Nuttall, Cobbett, and Strangeways-Pigg, Journal of Hygiene, 1901, I., p. 9.) Kerschbaumer[1] has, however, recently published some interesting data for the locality of San Pelagio in Istria. From May 26 to August 6, 1900, there were captured of the species Culex pipiens 2,787 females and 91 males; of C. maculatus, 2 females; and of Anopheles maculipennis (claviger) 572 females and 1 male. From August 8th to October 14th there were captured in horse-stables, carriage-houses, and hay-lofts 108 females and 42 males belonging to the species C. pipiens, and 1,667 females and 248 males belonging to the species A. maculipennis. In pig-pens there were found 2 females of C. pipiens and 646 females and 18 males of A. maculipennis. On the island of Hong Kong, out of thirteen localities tabulated by Thomson (Brit. Med. Journ., Sept. 14, 1901, p. 684), the proportion of Anopheles ranged from 0.2 per cent. to 56 per cent., the percentage of Anopheles captured in five localities being smaller than the percentage captured by me in Shelburne. MacGregor (Brit. Med. Journ., Sept. 14, 1901, p. 682) states that 70 per cent. of the mosquitoes that haunt the Government House in Lagos, West Africa, belong to the genus Anopheles.

THE LARVÆ OF ANOPHELES.

Methods of Collection. — I have employed with complete success the simple outfit recommended by Nuttall, Cobbett, and Strangeways-Pigg (op. cit., p. 12), consisting of a white enamelled dipper (the one used by the writer was 14 cm. in diameter and of 600 cc. capacity), a wide-mouthed pipette, and collecting bottles. The use of the white dipper made it possible to detect the larvæ very quickly and readily and to distinguish between Culex, Dixa, and Anopheles with the greatest ease.

---

[1] Fritz Kerschbaumer. Malaria, ihr Wesen, ihre Entstehung und ihre Verhütung. Vienna, Braumüller, 1901, pp. 89–95.

List of Localities in which Anopheles Larvæ were found.— In the following table the abbreviations p. and m. in the column headed "species" signify respectively the species A. punctipennis and A. maculipennis. When the letter is in brackets the identification is based on the examination of larvæ only. The height above sea-level is taken from the sheets of the U.S. Geological Survey.

ANOPHELES LOCALITIES.

PLACE.	Ht. above sea-level.	Dates when Anopheles larvæ were collected.	Species.	Notes.
Shelburne, N.H. (1)	700 ft.	June 25 " 26 " 27 " 28 " 29 July 2 " 4 " 7 " 10 " 16	p. m.	Ditch in intervale near Androscoggin River, 1–2 ft. deep. Sluggish current. No shade. Many tadpoles. About 300 yds. from house occupied by the writer and 70–100 ft. lower. Adults of A. punctipennis and A. maculipennis raised.
		July 18 " 22 " 24 Aug. 3 " 12 " 24 Sept. 1 " 7 " 18 " 19 " 21		C. stimulans found July 18, and later (Aug. 3) found small Dixa larvæ (also Aug. 22).
Shelburne (2)	700 ft.	June 30 July 1 " 25 Aug. 9	[p]	Ditch in intervale. No current. June 30 found 1 larva to about 3 dips. Made a second visit July 1, and caught only 2 larvæ in about 25 dips. No shade. Little life of any kind. Several predatory larvæ. Visited again July 25, and found nearly dry. 1 A. larva and a few small C. larvæ (stimulans). Completely dry Aug. 28.

Anopheles Localities. — *Continued.*

Place.	Ht. above sea-level.	Dates when Anopheles larvæ were collected.	Species.	Notes.
Shelburne (3)	800 ft.	July 8 " 9 " 13 " 22 " 24 Aug. 8	[p]	Roadside pool. 1 dip gave 2 A. larvæ, 2 C. larvæ (pungens), and 1 Dixa larva. Shaded densely during most of day. Few tadpoles. Spirogyra abundant. A. larvæ never abundant.
Shelburne (4)	900 ft.	July 8	sp?	Small pool in hollow of rock filled with rain water. About 5 in. deep and 6 in. wide, 3 ft. long. About 1 A. larva and 1 C. stimulans larva per dip. C. stimulans very abundant in other pools close by. July 22 visited again and found it completely dry. Found small Anopheles larvæ with Culex in neighboring pool in the rocks. Only C. in this pool August 1.
Shelburne (5)	725 ft.	July 26 Aug. 8	sp?	Margin of small pond about 100 yds. in diameter. Many Culex (pungens) — only 2 A. larvæ in about 40 dips. Tadpoles. Nuphar advena.
Shelburne (6)	740 ft.	July 29	sp?	Roadside pool, grass-grown, shaded. Little life. In 10 dips 1 A. larva, 3–4 C. sp(?), 15 Dixa. Evidently much extended by recent heavy rain.
Shelburne (7)	720 ft.	Aug. 12	[p]	Margin of small pond about 300 yds. in diameter. South bank of Androscoggin. Water very deeply colored with peat. Unshaded. C. pungens and C. stimulans abundant. 3 A. larvæ per dip.

ANOPHELES LOCALITIES. — *Concluded.*

PLACE.	Ht. above sea-level.	Dates when Anopheles larvæ were collected.	Species.	Notes.
Shelburne (8)	720 ft.	Aug. 12	sp?	Roadside pool near (7). Grass-grown. Unshaded. C. pungens and Dixa found also.
Gorham, N.H. (1)	800 ft.	July 3	p.	Stagnant pool near Androscoggin River. About 6–8 in. deep. Larvæ very abundant, especially among grass. 5–14 per dip. Pool partly shaded by large tree in afternoon. Few surface algæ (filamentous). Adult raised. Visited again July 25, and found completely dry. A little water on August 16, but no A. larvæ.
Gorham (2)	800 ft.	Aug. 16	sp?	Ditch in meadow near (1). Grass-grown. 2–3 larvæ per dip. C. pungens also.
Gilead, Me. (1)	720 ft.	July 29 Aug. 9	p?	Small pond in meadow. Grass-grown. Tadpoles. Nuphar. 1 A. larva per dip. About 20–30 (C. pungens) larvæ per dip. Some Lemna.
Gilead, Me. (2)	700 ft.	Aug. 9	p?	Meadow pool. Grass-grown. 1–4 A. larvæ per dip. No Culex.
Randolph, N. H. (1)	1,270 ft.	Aug. 14 Aug. 30	p.	Small ditch in meadow near Israel River. A. larvæ very abundant, 5 to 21 larvæ per dip. 10 pupæ captured. Spirogyra. Tadpoles. Much floating alga. 1 C. stimulans, a few Dixa. Adult raised.

The Relative Abundance of Larvæ. — The number of larvæ varied greatly in the different localities and in the same locality at different times. The smallest number of Anopheles

larvæ found in any place where they were found at all was in Shelburne (6), where Anopheles larvæ were so scarce that on the average only one was found in about twenty dips; in this locality at the same time the larvæ of C. pungens were very abundant, averaging forty to one hundred to a dip. The other extreme was Randolph (1), where as many as twenty-one Anopheles larvæ were found per dip. The extreme of variation in one and the same collecting ground was observed in Shelburne (1), where on September 7th as many as twelve per dip were found, and on July 14th only one was brought up in about five dips. In this locality the vicissitudes of collection are indicated by the following field notes:

June 25. "Very abundant."
June 26. "About 3 per dip."
June 27. "About 6 to 7 per dip."
June 28. "Abundant."
June 29. "1 to 4 per dip."
July 2. "1 per dip."
July 4. "3 to 9 per dip."
July 7. "1 per dip."
July 10. "1 to 2 dips."
July 14. "Very scarce," 3 larvæ in 15 dips.
July 18. "Very scarce," 7 larvæ in 25 dips.
July 22. "Very scarce," 1 larva in 3 dips.
July 24. "Very scarce."
Aug. 3. "Very scarce." Only 27 larvæ in half-hour.
Aug. 12. "More abundant than on Aug. 3."
Aug. 24. "Much more abundant than for some weeks, 35 larvæ in 15 dips."
Sept. 1. "24 larvæ in 10 dips."
Sept. 7. "38 larvæ in 10 dips."
Sept. 18. "36 larvæ in 10 dips."
Sept. 21. "32 larvæ in 10 dips."

Numerical estimates of this sort are somewhat fallacious, owing to inevitable irregularities of distribution, caused by winds, growth of water-plants, etc., but probably have some

value. It is important, however, that each observer at least should always follow substantially the same method of collection in his own work, since a slight divergence in the manner of "dipping" will lead to a difference in the number captured "per dip." The writer attempted to use in his work a uniform method, consisting in just immersing the lip of the dipper below the surface of the water and then swinging it rather rapidly through an arc of two to three feet, so that the surface quarter-inch of water would rush into the vessel.

The Length of Larval and Pupal Life.[1] — Although freshly reared female Anopheles were confined in breeding jars under what appeared to be favorable conditions and were fed with human blood, — and also with bananas and blueberries, — I did not succeed in getting them to lay eggs in captivity, and so did not secure data bearing directly upon the length of larval life. Very young larvæ — 1.5 mm. to 3 mm. — captured in the open did not for some undiscovered reason thrive very well under the conditions in which I kept them, and almost invariably died before reaching the pupal stage, in spite of frequent changing of the water and the addition of abundant supplies of water-weed. The older larvæ fared somewhat better, and in the course of the season twenty larvæ were transformed into pupæ and hatched. In no case, however, did the Anopheles larvæ prove as hardy as Culex larvæ under observation at the same time and under the same conditions. Anopheles larvæ were not as a rule kept alive for more than two to three weeks, whereas larvæ of C. stimulans and C. triseriatus could be kept alive almost indefinitely with little care, and in one case full-sized Culex larvæ (C. triseriatus) were kept in water containing little or no food for twenty-five days and pupated normally at the end of that time, normal adults developing from these pupæ.

In the open, more small larvæ than large ones are captured. At different times during the season, three hundred

[1] All statements made in this paper refer to A. punctipennis unless A. maculipennis is expressly mentioned.

and fifty-four larvæ were captured in eight localities in Shelburne. Measurements showed that two hundred and thirty-four of them were between 1 and 4 mm. and one hundred and twenty were between 4 and 8 mm. long. This falls in line with the observations of Nuttall and Shipley[1] in Cambridgeshire, England, so far as the excess of small larvæ is concerned, although in Shelburne the large larvæ were relatively more numerous than in England (34 per cent. of large larvæ in Shelburne, 22 per cent. in Cambridgeshire). Wide variations are noticed in the size of larvæ captured at different times in one and the same locality. This is illustrated in the notes of collection at locality Shelburne (1). (The actual numbers at the different dates have no significance, since the time spent collecting on the different days was not uniform.)

SIZE OF ANOPHELES LARVÆ.

Shelburne (1).

	1 to 4 mm.	4 to 8 mm.	
July 2	5	4	
" 4	14	10	
" 7	2	3	2 pupæ
" 10	2	7	
" 14	0	3	
" 18	5	4	
" 22	8	8	
" 24	0	3	
Aug. 3	23	4	
" 12	10	12	1 pupa
" 24	27	8	
Sept. 1	18	6	
" 7	29	9	1 pupa
" 18	29	7	1 pupa
" 21	21	11	
	193	99	

[1] Journal of Hygiene, 1901, I., p. 70.

The proportion of pupæ to larvæ was very low. In Shelburne (1), as the table shows, only five Anopheles pupæ were found in the entire season, as against two hundred and ninety-two larvæ collected in the same locality. The only other locality where pupæ were found was Randolph (1), where ten pupæ were collected on August 14. Larvæ, however, as well as pupæ, were more abundant in this latter locality than in any other place where collections were made.

The Anopheles pupa can be readily distinguished from that of Culex by the difference in position when resting at the surface of the water, as pointed out by Howard (Notes on the Mosquitoes of the United States. U.S. Dept. of Agriculture, Divis. of Entomology, Bulletin No. 25, p. 40). They are sometimes quite green[1] when young, but always darken on approaching maturity. The aquaria in which the larvæ were placed were examined at frequent intervals between 6 A.M. and 9 P.M., and in several instances the hour of pupation was determined with considerable precision. The pupæ were removed to tumblers of clear water immediately after their transformation from the larval state. In the majority of cases, although by no means invariably, both pupation and emergence from the pupa occurred during the night. In experiments made with pupæ kept in the ice-box at a low temperature (8–13° C.), the pupæ of A. maculipennis persisted two days in the pupal state after those of A. punctipennis had hatched. The exact duration of the pupal stage of A. punctipennis was determined in seventeen instances, the longest pupal life being nine days at 8 to 13° C. and the shortest being thirty-six to forty hours at a temperature of 23–32° C. Eight hatched in thirty-eight to forty-eight hours, five in forty-eight to seventy-two hours, one in about seventy-two hours, and three, kept at an artificially lowered

---

[1] Kerschbaumer (op. cit., p. 79) states that the spring brood of Anopheles pupæ observed by him at San Pelagio were almost all green, but that he never encountered a green Culex pupa. All the young pupæ of C. pungens that were found in Shelburne, and those of C. stimulans from some pools, were quite as green as any Anopheles pupæ.

temperature, in six to nine days; one imago died in emerging from the pupa after forty-eight hours. Out of fourteen pupæ captured in the open, four hatched within twenty-four hours, five within forty-eight hours, two within sixty hours, one in four days, and two died during transportation.

Only one pupa of A. maculipennis was captured; the imago emerged sixty hours later; in addition, two larvæ of this species were captured, and the pupal stage lasted seventy-two hours (temp. 20–25° C.) and eleven days (temp. 8–13° C.) respectively. The observations of Grassi cited by Nuttall and Shipley (op. cit.), of Howard (op. cit., p. 40), and of Nuttall and Shipley (op. cit., p. 275) indicate that the pupal stage of this species averages at least three to four days, and, according to Howard (op. cit., p. 41), usually lasts longer than this (a minimum of five days in June!). In my own experiments with pupæ kept in the ice-box at a low temperature (8–13° C.), the pupæ of A. maculipennis remained two days longer in the pupal state than those of A. punctipennis. It would seem to be true, therefore, that the pupal stage of A. punctipennis is perceptibly shorter than that of A. maculipennis under similar conditions,[1] although further observations where material is abundant are needed to establish this point.

Sex of Mosquitoes Hatched in Laboratory. — Thirty larvæ and pupæ of A. punctipennis collected at intervals through the season gave birth to eleven male and nineteen female adults. There was on the whole a preponderance of female adults among the mosquitoes hatched out in the laboratory, although the numbers dealt with are not large.

---

[1] G. W. Herrick (Science, N.S., XIV., No. 348, Aug. 30, 1901, p. 330) states that the duration of the pupal stage of A. punctipennis, as observed by him in Mississippi, was two days (temperature of the water not given).

	Males.	Females.
A. Punctipennis	11	19
" *Maculipennis*	2	1
C. Stimulans	10	14
" *Triseriatus*	3	16
" *Impiger*	1	0
" *Pungens*	2	2
Aedes sp.	0	6
	29	58

Seasonal influence, if it exists, is not marked. It does not appear that any more males than females are hatched during the early part of the summer. The effect of temperature and other causes upon the proportion of the sexes should be made the subject of experimental work in localities where the material is sufficiently abundant, as the matter has obvious practical bearings.

Habitat, etc. — Anopheles larvæ were found in thirteen different localities — eight within the town limits of Shelburne, N.H., two in the adjoining town of Gorham, N.H., one in Randolph, N.H., and two in Gilead, Me. In most cases the collecting-grounds were visited several times during the course of the season. Nine out of the thirteen bodies of water remained permanent through the season, while four pools in which Anopheles was found breeding in the early summer dried up completely before the close of August.[1]

The season of 1901 was above the average in amount of rainfall in this region, and I think it probable that at least three more of the breeding-places would disappear in a dry year.

In eleven out of the thirteen localities Culex larvæ were found together with Anopheles larvæ; only a single collection was made in each of the other two. There were several

[1] One pool in which drying-up occurred became again filled with water later in the season, but no mosquito larvæ of any kind could be found in it.

localities, notably Shelburne (1) and (2), in which only Anopheles larvæ were found in the early part of the summer, no Culex larvæ appearing until later. In four places the Culex larvæ associated with Anopheles were those of C. stimulans, in five those of C. pungens, in one place both species were present, and in one case the species of Culex inhabiting the same pool with Anopheles was undetermined. It is to be noted as a fact not devoid of significance that wherever the larvæ of one genus were found very abundantly, those of the other genus were notably rare. Thus in the ditch where Anopheles larvæ were found in greatest numbers (Randolph (1)), only a single Culex larva was brought to light in fifty dips, whereas in Shelburne (5) only two Anopheles larvæ were captured in about fifty dips, the Culex larvæ (pungens) averaging at the same time thirty to forty per dip. In other cases, however, where neither genus was very plentiful, the two seemed to be on substantially the same numerical footing (cf. Shelburne (3) and (4)). It is doubtless true that the preponderance of one or the other genus in a particular breeding-pool is due not so much to instinctive selection of a spot for egg-laying by the female mosquito as to the presence or absence of conditions favorable for larval development. At all events, there can be no question that straggling Anopheles females sometimes lay eggs in bodies of water where the conditions are more suitable for the development of Culex larvæ than for that of their own young. Dixa larvæ were found in five of the Anopheles localities, in one instance (Shelburne (6)) in much larger numbers than either Anopheles or Culex. Anopheles larvæ thrive especially in unshaded waters, although three out of the thirteen localities in which I have collected were densely shaded. In the shaded pools, however, they were always found in comparatively inconsiderable numbers. Dixa is more abundant in shaded than in unshaded water.

The general character of those bodies of water in which Anopheles were found breeding most abundantly is a matter of especial interest. Examination of the table of localities will show that all the Anopheles pools possessed one character in

common. They were accumulations of water in ditches or pools in the intervale of the Androscoggin or in meadows bordering smaller streams. Such ditches were in some cases spring-fed, but in most cases they simply intercepted the ground water on its way to the river. In this respect they are quite homologous to the so-called filter-basins excavated near the bank of a stream for the purpose of capturing the ground water flowing towards the river. Many municipal water-supplies in the eastern part of the United States are derived from such filter-basins, which often furnish water of quite exceptional purity. The ditches in the intervale of the Androscoggin resemble in all essential respects these filter-basins. It is well-known that the storage of such ground-waters for public water supplies involves peculiar difficulties, in that waters which, like these, are rich in nitrate, afford highly favorable conditions for the development of microscopic algæ, when exposed to sunlight and air, and if undesirable forms get a foothold in such a water disagreeable odors and tastes are frequently produced. The amazing multiplication of microscopic forms in ground-waters of this class is conclusive proof of the superior character of such water as a pabulum and breeding-place for algal life. It is precisely upon these microscopic organisms that the well-being of Anopheles depends. The Anopheles larvæ, as is well known, are surface feeders and derive their sustenance from the plankton. In this respect they differ from most Culex larvæ, which are able to browse over the bottom and get their food in large part from the bottom ooze as well as from the floating life or plankton. It is no accident, therefore, that malaria has been observed to prevail especially along river bottoms and sea coasts. These are the very localities, at the bases of water-sheds, that are most likely to afford an opportunity for the ground-water to come into contact with light and air, and whenever this happens, Anopheles larvæ are provided with suitable and abundant food. Accumulations of surface water, on the other hand, are less apt to furnish proper pabulum, at least in temperate regions, and are available as breeding-places only for the larvæ of

those genera of mosquitoes that are able to range more freely in their search for food than Anopheles larvæ.

It need hardly be pointed out that not only the ditches and trenches in river intervales and meadows that fill with ground-water are favorable breeding-places for Anopheles, but also pools made by damming the flow from a spring or other ground-water source. This is unquestionably one reason why the excavations for railways, sewer-pipes, and water-pipes, which disturb the natural drainage channels and lead to the formation of quiet pools of spring (ground) water, are frequently followed by outbreaks of malaria. Pools formed of a sewage-polluted river water in which vitrification has occurred fall into the same category with other nitrate-rich water.

Influence of Temperature. — As has been already pointed out, pupation and emergence from the pupa, and presumably larval development likewise, take place more rapidly at a moderately high temperature than at a low. Pupal development, at least, is perfectly normal at a temperature of 26–32° C. At the same time, a marvellous degree of adaptability to low temperature is shown. One large larva of A. punctipennis pupated normally in the ice-box at 8° C., and a normal imago emerged six days later, the temperature of the water never ranging higher than 13° C. during this period and averaging about 10°. My last collection of Anopheles larvæ in the field was made on September 21, when the temperature of the air was 13.5° and that of the water 15°. At this time and for some days previously no Culex larvæ were found in this locality. In the whole region, in fact, there was a striking absence of Culex larvæ. In localities Shelburne (4) and (5), where early in the season there had been a great abundance of Culex larvæ (C. stimulans and C. pungens respectively), not a single Culex larva could be found on September 8, or again on September 19.

This falling off in the number of Culex larvæ at the approach of cold weather is in marked contrast to the persistence of numerous Anopheles larvæ in at least one of the localities under observation (p. 11).

Enemies. — Although no systematic studies were made, two aquatic forms were observed to prey upon Anopheles larvæ under natural conditions. On several occasions Anopheles larvæ were seen struggling in the grasp of these enemies immediately after the dip had been made and when the catch was first examined. These two enemies were the larval form of Dytiscus and a species — perhaps several — of Notonecta. Large Anopheles larvæ, also, would not infrequently devour their own kind in the aquaria, but were not observed to do this under more normal conditions.

A. Punctipennis. — Most of the statements in this paper refer only to the larvæ and pupæ of A. punctipennis. The larva of A. punctipennis can be most easily distinguished from the larva of A. maculipennis by the pigment markings on the dorsal aspect of the head. An excellent figure of A. maculipennis is given by Nuttall and Shipley (op. cit., Plate II., Fig. 4), but I have not been able to find anywhere a sufficiently detailed drawing of the A. punctipennis larva. The accompanying figure (Plate I., Fig. I.) may consequently be useful in enabling observers to separate the two species by larval characteristics.

A. Maculipennis. — I am unable to offer any explanation for the relative scarcity of A. maculipennis in this locality. Only three individuals of A. maculipennis were raised from larvæ and pupæ captured during the season (all from Shelburne (1)), as against thirty individuals of A. punctipennis from the same pools. No adults of this species were captured, as compared with six specimens of A. punctipennis. Curiously enough, A. punctipennis has not been recorded before from the State of New Hampshire, but the species A. maculipennis, which was so much less abundant in Shelburne and Gorham in 1901, has been recorded from the neighboring town of Berlin. (Howard, op. cit., p. 40.)

NOTE UPON OTHER SPECIES OF MOSQUITOES.

The mosquito fauna of Shelburne is quite large. Seven species in all have been collected — C. stimulans, C. impiger, C. triseriatus, C. pungens, A. punctipennis, A. maculipennis, Aedes sp.

C. Stimulans. — The mosquito most conspicuous through its aggressiveness during the season of 1901 was C. stimulans. Especially in the woods, this species was always ready to attack in large numbers, from the beginning of the season to the end. Of the mosquitoes captured in the act of attacking man it was numerically predominant. This species was found breeding in large numbers in the pools in rock hollows along a mountain brook, and also, but less abundantly, in small pools and ditches in the intervale. The larvæ are usually large, and hang nearly or quite vertically from the surface. They can remain below the surface without rising for as long as eight minutes. The pupal stage lasts from three to four days at 20 to 25° C. Fourteen females and ten males were hatched from larvæ and pupæ collected between July 8th and August 1st.

C. Triseriatus. — This species was quite abundant about the house early in the summer, and after some search their breeding-place was discovered in a pool of water that had gathered in a hollow in an elm tree about three feet above the ground. The water was a rich leaf infusion and had a deep brown color. A male and a female adult were captured in this hollow, and a number of larvæ and pupæ were collected on July 10th. Sixteen females and three males were hatched. The average duration of the pupal stage was four to six days at 20 to 25° C. The house was supplied with mosquitoes mainly from this tree, and the application of a few drops of kerosene to the pool diminished the number of this species invading the house and prevented all development in the tree during the remainder of the summer.

C. Impiger. — The adults of this species were occasionally collected during the early part of the season, but were encountered less frequently during the latter part. Very large larvæ and pupæ were found in a pool in deep woods on a mountain side about 2,200 feet above sea-level. They did not bear transportation well and only one male was hatched. Many of the individual mosquitoes captured in the early summer were infected with a small red mite kindly examined for me by Dr. L. O. Howard, who writes me that it is the immature stage and has not been bred, so that the adult is not known with certainty. It is at present known by the name of *Acarus culicis.*

C. Pungens. — This species was not often captured in the neighborhood of the house, but was sometimes met with along the road and was found breeding in large numbers in several localities. It was found with Anopheles in locality Shelburne (6); four pupæ taken here gave rise to two males and two females. The breathing-tube in this species is proportionately much longer than the breathing-tube of the three other species of Culex observed in Shelburne. The head is larger in proportion to the body and the larva itself smaller than the other species. As Howard has stated, this species does not remain long under water, — another respect in which it differs from the other species which I had under observation.

Aedes sp. — This species appear to differ from Aedes fuscus, according to the published figures (Howard, Mosquitoes, McClure, Phillips & Co.). It was found breeding in pitcher-plant leaves growing around a small pond at the edge of the intervale and also in pitcher-plants on the margin of a small mountain lake 2,500 feet above sea-level. Not very numerous when first found (July 12th), but later in the season (July 27th–August 5th) nearly every leaf contained a pure culture of the larvæ. Three pupæ were found on July 27th. The larvæ are very translucent and very different in character from any other mosquito larvæ

that I have seen figured. Both larvæ and pupæ are able to remain under water much longer than the young of other mosquitoes. The larvæ can apparently stay below the surface indefinitely; they certainly so remain for hours at a time. The specific gravity of the pupæ seems somewhat greater than that of other pupæ that I have observed. I have timed one pupa below the surface of the water for five minutes. Six females have been hatched. The larvæ are more hardy than those of any other species I have had under observation. They bear transportation perfectly and will remain alive for months (as long as one hundred and sixty-two days at 20° C.) without any other care than that of occasionally adding water to the tumbler to prevent drying up. The pupal stage lasts five to eight days at 20–25° C. The larvæ are negatively heliotropic to a marked degree.

JORDAN. ANOPHELES PUNCTIPENNIS.

IODOPHILIA.

EDWIN A. LOCKE, M.D., AND RICHARD C. CABOT, M.D.

A Preliminary Report.

The material for this preliminary communication has been furnished by cases which we have examined in the Out-Patient Department and wards of the Massachusetts General Hospital during the past four months.

Iodophilia, so called, is the reaction which certain of the white cells of the blood show when a dried blood-film is brought in contact with a drop of the following solution:

Iodine,	1 gram.
KI.,	3 grams.
Water,	100 c.c.
Gum arabic,	50 grams.

This mixture was first suggested by Ehrlich in 1883, but the capacity of the polynuclear leucocytes, especially in pus, for reaction to iodine has been known for more than twenty years. It has not, however, come into general use as a means of clinical diagnosis, and is but little used to-day. This is but natural, for the observations of different investigators have in many cases been so varied that its clinical applications have never been definitely established.

Our purpose is to make clear first of all just what the reaction is, and then, after a sketch of the literature, briefly to summarize our own results in its use.

The technic is simple. A cover glass film is prepared in any of the usual ways, and allowed to dry in the air. Without fixation it is then pressed down upon a drop of the iodine solution on a slide and examined with an oil immersion lens. In making the preparation, it is well to use slightly more of the mixture than is necessary to cover the film, in order that the cells may all come freely in contact

with the iodine, and then to squeeze out the excess by gentle pressure on the cover glass lest the dense color of the fluid obscure the field. When such a slide of normal blood is examined, the red cells are found to be uniformly colored a bright yellow upon a much fainter background, while the white corpuscles are stained of about the same tint, their nuclei being somewhat more refractile. This contrast between the nucleus and protoplasm is sufficiently distinct to permit one readily to differentiate the various forms of white cells. In certain pathological conditions, such as septicemia or uremia, the uniform yellow coloration is broken by the appearance in the protoplasm of the polymorphonuclear neutrophiles of reddish-brown granules, or a diffuse brownish coloration, and by the presence of small and large masses outside the corpuscles similarly colored. This condition is the "iodine reaction," and as suggested is of two distinct types, namely, 1, the extra-cellular, and 2, the intra-cellular.

1. Extra-cellular reaction:

The masses seen outside the cells are round, oval, or slightly irregular, varying in size from two to six, or even eight mikrons in diameter, and of a copper red color. They may be free, but are usually found in fragments of protoplasm which appear to be the débris of broken-down leucocytes.

2. Intra-cellular reaction:

Within the cells the reaction occurs almost invariably in the neutrophiles. Here the appearance is extremely varied, the granules being of almost any depth of color from a light orange to the deepest brown, and of various sizes. They may be scattered evenly throughout the protoplasm, grouped about the nucleus, or, in rare cases, at the periphery of the cell. In sharp contrast to the round or oval extra-cellular bodies, those within the cells are for the most part irregular in shape. In many of the neutrophiles the reaction is shown not by a granular appearance of the protoplasm, but by a diffuse brownish discoloration, the depth of which varies within wide limits. In rare cases basophiles and myelocytes react, but here the picture is a different one, for instead of many granules scattered throughout the protoplasm, one

finds a more or less distinct single row of relatively large brownish areas about the nucleus. We have never observed any brown color, either diffuse or granular, in the eosinophiles.

It is important to note that in some specimens only a few neutrophiles are found which are abnormal. The intensity of the color appears to be of as much importance as the number of corpuscles showing it. Not infrequently a large number of leucocytes are seen absolutely unaffected by the iodine solution before any are found with the characteristic coloration. With other cases scarcely any normal ones can be demonstrated. In our examinations we have, as a routine, counted at least one hundred cells; if in that number none are observed with either a diffuse or granular stain, we consider the reaction negative.

The appearances of the brown stained leucocytes are shown in the plate.

The history of this reaction is very briefly as follows:

Ehrlich,[2] in 1883, called attention to its occurrence especially in pus. Gabritschefsky,[3] in 1891, made a thorough study of the reaction, both in normal and pathological blood, and figured with the greatest accuracy almost all of the appearances which have since been described. Like all later observers, he has failed entirely to find any intra-cellular reaction in normal blood, but occasionally found a few of the extra-cellular masses in the blood of healthy individuals. This has been confirmed by the results of most later observers. Believing as he did that the reaction was due to glycogen, an assumption made by Ehrlich and generally credited at that period, Gabritschefsky studied especially cases of diabetes in which he found a considerable reaction, both within and without the cells. He had a similar experience in leukemia, but, although he studied many other diseases in which other observers have later found the reaction present, he was unable to find any within the cells, except in the two diseases already mentioned. By animal experimentation he found that he could produce marked intra- and extra-cellular reaction in the blood of dogs by introducing large amounts of

carbohydrates or of peptone, either into the stomach and peritoneal cavity or the jugular vein. In diabetes produced in dogs by the excision of the pancreas, the reaction was well marked, while in experimental phloridzin diabetes it did not occur or only to a very limited extent.

In 1893 [1] Czerny investigated the subject in the Children's Clinic with the following results. In normal infants, one to six years of age, no reaction of any kind. In disease the reaction was well marked within the polynuclear leucocytes in the following conditions:

1. Infantile atrophy complicated by anemia, furunculosis, or lobular pneumonia.
2. Chronic tuberculosis of bone or lungs.
3. Abscess or septicemia of the new born.
4. Lobular pneumonia, especially during the later stages when the leucocytosis is falling.
5. Congenital atelectasis with marked dyspnea.
6. Hemophilia.

Experimentally Czerny was able to produce the reaction in dogs by exposing them to a temperature of two degrees Centigrade for twenty-four hours, either by narcosis, by artificial dyspnea produced by means of an operative pneumothorax or by bleeding, and through the production of leucocytosis by the injection of turpentine. He concludes that the three elements most concerned in the production of the reaction are:

1. Disturbances of respiration.
2. Anemia.
3. Pus.

Czerny raises a question regarding the nature of the substance in the leucocytes which react to iodine, and points out the various other substances, *i.e.*, amyloid, which give similar reactions. He does not, however, definitely decide to what substance the reaction is due.

In 1894 Livierato [9] made a very thorough study of the reaction both in health and disease. He found it present in all cases of pneumonia and empyema, in about one-third of the cases of typhoid, in advanced febrile cases of phthisis, and

in a single case of each of the following diseases: measles, scarlatina, diabetes, splenomegaly, malaria, pyemia, cancer of colon with ulceration.

Negative in three cases of rheumatism, three of jaundice, several of heart disease in all stages, and also in single cases of grippe, lead colic, amyloid liver, and chronic tubercular peritonitis. In the last half of the nine months of pregnancy and for a few days after delivery, he found the reaction frequently positive.

Goldberger and Weiss,[4] as a result of the study of cases with abscess, conclude that the reaction is present in all advancing suppurative processes, and that its intensity is proportional to the rapidity of the purulent accumulation. Non-progressive, "stationary" accumulations of pus do not give rise to any iodophilia.

After an abscess has been opened the iodophilia diminishes, but does not altogether disappear for several days despite free drainage (*e.g.*, for six days after drainage of abscess near the elbow). During this period the brownish color is apt to appear as if oozing out at the extreme periphery of the leucocyte in the form of bead-shaped masses or narrow bands, while the rest of the protoplasm is entirely colorless. In the earliest stages of inflammatory infiltration before fluctuation is detected there is usually no iodophilia.

The size of the abscess makes no difference. Felons with only a thimble full of pus may be associated with as intense an iodophilia as chronic abscesses the size of a child's head. Indeed, abscesses of the latter type, if tubercular or "cold," often show no iodophilia at all.

In acute exacerbations of dormant abscesses the reaction promptly reappears.

The writers agree with Czerny that a reaction, lasting perhaps twenty-four hours, may be produced by prolonged narcosis, and by disturbances of respiration (due to cerebral edema, or terminal dyspnea) as well as by pneumonia.

Extra-cellular masses staining brown with iodine are found in varying amounts in normal as well as in pathological blood. They are often increased after fracture, operations

on bones, and the subcutaneous effusion of blood (hematoma), the increase usually appearing about twenty-four hours after the injury. Occasionally an intra-cellular iodophilia with "aseptic fever" appears two to three days after the injury.

The writers believe the extra-cellular iodophilia to occur in the débris of dead leucocytes, and attempt to prove their assumption by experimental work. Increased extra-cellular reaction is accordingly interpreted to mean increased destruction of leucocytes.

Kaminer[7] practically agrees with Livierato and Czerny in most of his results. He found positive reaction in all kinds of sepsis with leucocytosis, including puerperal sepsis with or without localization, and in the earlier stages of all cases of pneumonia. Like Livierato he failed to get any reaction in rheumatism, and never obtained it in cancer or phthisis, unless secondary infection and ulceration had taken place. Out of five cases of diabetes, four were negative and the fifth (in coma) was positive. In this last case there was at the time a marked leucocytosis.

He failed to find the reaction in cancer (with or without leucocytosis), many cases of chlorosis and secondary anemia, as well as in two cases of leukemia, and one case of amyloid disease with leucocytosis.

In one case of Werlhof's disease with chronic hemorrhagic nephritis, a well-marked reaction occurred.

To Kaminer the factors effective in producing the reaction appeared to be:

1. Fever.
2. Leucocytosis.
3. Toxemia.

But since artificial leucocytosis (due to spermine) and artificial fever (produced by the "fever puncture") had no effect upon the leucocytes, Kaminer's attention became concentrated on toxemia, the third of the factors suggested by his clinical study. Accordingly he injected twenty animals with diphtheria toxine and produced an intra-cellular iodine reaction in fourteen. Of the positive cases all but one had

fever. Of those without fever (five), only one showed a positive reaction.

Hofbauer [5] studied seventeen cases of severe chlorosis, eight of them having thirty-five per cent. or less of hemoglobin, and got no positive intracellular reaction. Dark yellow tints in the polynuclear leucocytes are often mentioned, but nothing more distinctive. In secondary anemia of moderate grade, he found but two positive reactions among eighteen cases. Severer cases of secondary anemia (seven) showed marked reaction, despite the absence of leucocytosis and even with leucopenia (1,200 per mm.).

In five cases of pernicious anemia the reaction was present from time to time, especially near death.

Among the nine cases of leukemia studied by Hofbauer, six were myelogenous and three lymphatic in type. Intracellular iodine was present in all of them, though in but very few cells. The author makes no mention of a reaction in any but polynuclear cells. The reaction was much more marked in the acute cases than in the chronic.

In pseudo-leukemia (seven cases) no reaction was found, but in cases of tuberculosis of the liver and spleen, with clinical symptoms and signs closely resembling pseudoleukemia, the iodine reaction was well marked.

In one case of purpura hemorrhagica no reaction was found.

Hofbauer considers the occurrence of an iodine reaction in the leucocytes of cases of anemia as a bad prognostic sign.

He points out that the reaction is in no way dependent on the presence of leucocytosis, since in many of the cases studied by him the number of leucocytes was normal or diminished.

Our own studies with the reaction include four hundred and thirty-two cases. In the following table we have attempted, as briefly as possible, to summarize our results classifying the intra-cellular reaction as positive or negative merely, and noting the extra-cellular only when increased.

TABLE I.

DISEASES.	No. cases.	Negative intra-cellular.	Positive intra-cellular.	Increased extra-cellular.	Remarks.
Abscess	14	1	13	1	Negative case; a small stitch-hole abscess.
" opened, thoroughly drained	8	8			
" lungs	2	....	2	1	
Actinomycosis, cervical abscess	1	....	1	1	10,000 leucocytes.
Alcoholism, chronic	4	4			
Anemia:					
Pernicious A	6	5	1		
Chlorosis	1	1			
Secondary:					
Trauma with severe hemorrhage	2	1	1	1	[1] Whites 19,000.
Gastric ulcer	2	1	1	....	[1] Hgb. 28% Erythrocytes 1,960,000. Leucocytes 28,000. [2] Hgb. 22%. Erythrocytes 2,000,000.
Malignant disease	1	...	1		
Aneurism	1	1			
Asplenia	1	1	....	....	Spleen removed 6 mos. previous.
Appendicitis:					
Chronic	5	5	....	....	All without pus or severe inflammation.
Acute, without local abscess	9	....	9	2	
" with local abscess	11	....	11	1	
Interval	1	1			
Diagnosis A., but found normal	2	2			
Asthma, bronchial	11	7	4	2	
Arterio-sclerosis	1	1			

TABLE I. — *Continued.*

DISEASES.	No. cases.	Negative intra-cellular.	Positive intra-cellular.	Increased extra-cellular.	Remarks.
Blisters, multiple	1	1			
Bone lesions:					
Osteomyelitis	1	....	1	....	Extensive.
" after free drainage	6	6			
Brain tumor	2	2	....	1	
Bronchitis	11	6	5	2	
Bubo	1	....	1	....	12,000 leucocytes.
Burns, universal	1	1	....	...	78,000 "
" hand, by electricity	1	1	....	....	8,000 "
Carbuncle	1	....	1		
" after excision	2	2	....	....	[1] 2¼ days; [2] 1 week.
Cardiac:					
Aortic disease	1	1			
Cardio-renal	1	1			
Mitral disease	5	3	2	1	All with broken compensation.
Myocarditis	2	2	....	....	With edema.
Carbon monoxide poisoning,	1	1	....	....	20,000 leucocytes.
Cirrhosis liver	2	2	....	....	Probably alcoholic.
Constipation, obstinate	4	4			
Cystitis	1	....	1	....	Very severe.
Debility	4	4			
Diabetes Mellitus	7	6	1	6	
Empyema	5	....	5		
Enteritis	6	1	5		
Exophthalmic goitre	1	....	1	....	Complicated by bad cardiac condition.

TABLE I. — *Continued.*

DISEASES.	No. cases.	Negative intra-cellular.	Positive intra-cellular.	Increased extra-cellular.	Remarks.
Extra-uterine pregnancy with rupture	2	2	....	....	[1] 34,000 leucocytes. [2] 54,000 "
Extra-uterine pregnancy unruptured	1	1	....	....	9,000 "
Furunculosis	1	....	1		
Fibroid	1	1			
Gallstones	7	2	5		
Gangrene leg	1	....	1	1	40,000 leucocytes.
Toe, superficial	1	1	....	1	Head injury, 5% sugar.
Fingers	1	1			
Gonorrheal arthritis	1	....	1	....	17,000 leucocytes.
Heat prostration	1	1			
Hernia:					
Inguinal	1	1			
" strangulated	4	2	2	1	
Herpes Zoster Brachialis	1	1	....	1	Very extensive.
Hodgkins' disease	2	1	1		
Intestinal obstruction	4	2	2	2	2 positive cases near death.
Lead poisoning	4	4			
Lumbago	1	1			
Leukemia:					
Lymphatic	1	1			
Myelogenous	2	....	2		
Malaria	48	29	19		
Malignant disease:					
Cancer	16	7	9	2	
Epithelioma lip	1	1	....	1	

TABLE I. — *Continued.*

DISEASES.	No. cases.	Negative intra-cellular.	Positive intra-cellular.	Increased extra-cellular.	Remarks.
Malignant disease, *cont.*:					
Lympho-sarcoma	1	....	....	1	Intestines.
Melanotic sarcoma	1	....	1	....	Near death.
Sarcoma	4	4			
Melanoderma Lenticularis Progressiva..............	1	1	....	....	Very extensive. Normal leucocyte count.
Nervous dyspepsia	1	1			
Nephritis:					
Acute	3	....	3	1	
Chronic.................	2	....	2	1	
Uremia	3	1	2		
Neurasthenia	5	5			
Otitis Media...............	1	....	1		
Ovarian cyst	1	....	1	....	? of complicating local sepsis.
" " with twisted pedicle ...	1	1	....	....	40,000 leucocytes.
Peritonitis:					
Pelvic	2	....	2		
General.................	13	....	13	5	One case with 4,800 leucocytes. " " " 8,000 "
Phlebitis	1	....	1		
Pleurisy:					
Chronic dry pleurisy......	3	3			
P. with effusion	6	6			
Pleurodynia	1	1			
Pneumonia:					
Broncho	1	....	1		
Central	1	....	1		

TABLE I. — *Continued.*

DISEASES.	No. cases.	Negative intra-cellular.	Positive intra-cellular.	Increased extra-cellular.	Remarks.
Pneumonia, *continued:*					
Influenza	1	....	1		
Lobar	13	....	13	2	
Pregnancy	1	....	1	....	7th month.
Renal colic	1	1	....	....	7,200 leucocytes.
Rheumatism:					
Acute articular	5	5			
Subacute	2	2			
Ruptured uterus	1	....	1	1	20,000 leucocytes, with general peritonitis, near death.
Salpingitis	7	....	7	2	All with pus or severe inflammation.
"	2	2	....	....	Only very slight inflammation.
Septicemia	8	....	8	4	3 cases malignant endocarditis.
Septic leg	1	....	1		
Septic wounds after operation	6	3	3		
Shock	1	....	1	....	Arm torn off, 3 hrs. after accident. 30,000 whites.
Syphilis, liver	1	...	1		
Secondary	1	1	....	1	
Tape-worm	1	1			
Thrombosis, mesenteric	1	....	1	1	3 ft. necrotic intestines, 24,000 whites.
Pulmonary	1	....	1	1	With phlebitis. Near death. 26,000 whites.
Tonsillitis	1	....	1		
Tuberculosis: Miliary	1	....	1		
Abscess liver	1	1	....	1	
Bone	4	4			
Glands	4	1	3		
Kidney	2	....	2		

TABLE I. — *Concluded.*

DISEASES.	No. cases.	Negative intra-cellular.	Positive intra-cellular.	Increased extra-cellular.	Remarks.
Tuberculosis, *continued:*					
Peritonitis	6	4	2		
Phthisis	11	11			
" with secondary infections	4	....	4		
Typhoid	23	14	9	1	
Vaccination	5	3	2		
Vaccinia	1	....	1		
Varicose ulcer	1	1			

DIAGNOSTIC VALUE OF IODOPHILIA.

The following cases will illustrate the practical application of this blood reaction in differential diagnosis:

Case I. — T. S.; Sept. 19, 1901; 37 yrs.; accident room.

Abdominal pain beginning in right side and becoming general, with vomiting for four days. Bowels moved each day with cathartics.

P.E. — Facies suggests severe septic condition. Abdomen board-like with general tenderness, which is more marked on right.

Temp., 103° F.; pulse, 104; resp., 30.

White count, 4,800; Iodophilia very marked.

Operation revealed a general peritonitis.

Case II. — J. A. H.; Sept. 22, 1901; 13 yrs.; accident room.

Brought to accident room with four days' history of severe abdominal pain and vomiting. Temperature normal. With hot poultices and cathartics considerable relief, but five hours ago suddenly became worse.

P.E. — Very cyanotic; looks sick. Abdomen much distended, very hard and tender. Pulse rapid and weak.

Temp., 104° F.; pulse, 165; resp., 30.

Whites, 8,000. Iodophilia very marked.

Operation: General peritonitis found.

Case III. — A. S.; Aug. 27, 1901; 20 yrs.; accident room.

Three days previously onset of severe paroxysmal abdominal pain which became more or less localized on right side. Vomited on second day. No chills. Bowels constipated.

P.E. — Abdomen rigid, slightly more marked on right with considerable tenderness. No masses felt. Rectal examination negative.

Temp., 98.4° F.; pulse, 120; urine negative.

Whites, 15,000. Iodophilia negative.

Operation: Appendix and pelvic organs normal.

Case IV. — D. N. C.; Sept. 23, 1901; 31 yrs.; accident room.

Indefinite symptoms for one week. Yesterday taken with very severe cramp-like abdominal pain: morphia. No vomiting.

P.E. — Abdomen somewhat distended, with marked resistence and considerable tenderness on right. Urine negative.

Temp., 99.5° F.; pulse, 60; resp., 20.

Whites, 14,000. Iodophilia negative.

Operation: Appendix removed. Pathological report, "normal appendix."

Case V. — M. C.; Aug. 11, 1901; 31 yrs.; accident room.

For five weeks not feeling well. More or less pain in lower abdomen, at times sharp and cramp-like. During past three days pain more intense and great prostration, frequent vomiting, bowels constipated, marked thirst. Catamenia regular. No signs of pregnancy.

P.E. — Extreme pallor. Abdomen tense, tympanitic,

generally tender, no dullness in flanks. Vaginal examination: large indefinite mass felt in left cul-de-sac; considerable general tenderness.

Temp., 100.8° F.; pulse, 112; resp., 36.

Whites, 32,000. Iodophilia negative.

Operation: Ruptured extra-uterine pregnancy found.

Case VI. — J. J. McD.

Fifteen months before entrance to hospital was kicked in left thigh by horse. Resulting swelling, great tenderness, and severe pain. Unable to work for thirteen weeks. For past four months increased pain in same region with considerable tenderness.

P.E. — In outer side of left thigh a small fusi-form swelling felt deep under muscles, apparently attached to femur. Moderately tender. X-ray shows slight evidence of periostitis.

Temp., 99.6° F.; pulse, 70; resp., 20.

Whites 10,000. Iodophilia negative.

Operation: Only slight thickening of periosteum found.

Case VII. — C. S.; Sept. 5, 1901; 21 yrs.

Six days history, cough, high fever, occasional chill, dyspnea, pain in right chest, anorexia, and great weakness.

P.E. — Cyanotic, shallow breathing. Lungs: right expands slightly less than left. Below fourth rib, and two fingers below angle of scapular on right, dulness with distant bronchial breathing, distant voice sounds, absent fremitus. Interspaces alike on two sides. Above dull area respiration harsh, many moist rales.

Temp., 104° F.; pulse, 125; resp., 30.

Whites 9,000. Iodine negative.

Two days later tapped and ℥ 24 fluid drawn off. Pleurisy with effusion.

Case VIII. — R. B.; July 22; 37 yrs.

Three months' history, following probable pneumonia, of persistent cough, weakness, pain in left side of chest with moderate dyspnea. No chills, fever, or sweats.

P.E. — Slightly pale, but no cyanosis. Lungs: right clear; movement of left somewhat diminished; flatness at base to within two fingers of angle of scapular and sixth rib anterior axillary line; over this area absent tactile fremitus, much diminished respiration and voice. No change in signs with change in position. Lungs otherwise normal.

Temp., 98.4° F.; pulse, 120; resp., 27.

Whites 10,000. Iodophilia very marked.

Tapped, and after third attempt pus found. Empyema.

From our observations thus far we feel justified in the following conclusions:

The brown masses outside the corpuscles are present in small numbers in all blood both normal and abnormal, but frequently in such minute amounts as to be found only after long searching. Only an increase of these masses is to be considered pathological. We have found them uniformly and markedly increased in diabetes mellitus, variably in chronic or very severe acute suppurative diseases, and rarely in a few other conditions. In general we may say, from our present knowledge, that the increase of extra-cellular iodophilia is of little significance.

In sharp contrast to the extra- we have never seen the intra-cellular granules in the blood of normal individuals, and believe its presence always pathological. Hence, by the terms positive or negative reaction, we make reference alone to the latter.

1. Like leucocytosis, fever, and the diazo reaction, iodophilia signifies not a special disease or condition, such as abscess, but a general toxemia such as might be produced by abscess, gangrene, uremia, or malaria. Though more constantly positive in the presence of pus than with other conditions, we cannot make a diagnosis of sepsis, or of a purulent accumulation from the reaction alone.

2. Iodophilia is not identical with, neither does it coincide in its indications with, any of the ordinary physical signs, as leucocytosis, fever, etc. (see table).

3. It appears to be certain evidence that the patient is

sick. We have never observed the sign in any but severe cases, and believe it to be more reliable in this respect than either leucocytosis or fever.

4. A positive reaction occurs with considerable regularity in the following conditions:

a. Infection with pyogenic organisms, whether local or general.

b. Toxemia of bacterial origin, as in diphtheria and typhoid.

c. Non-bacterial toxemia; *e.g.*, uremia.

d. Disturbances of respiration.

e. Grave anemia, both primary and secondary.

5. In our experience the sign has been absent in pleurisy, rheumatism, extra-uterine pregnancy, alcoholism, abscesses with free drainage, lead poisoning, early malignant disease, nervous conditions, tuberculosis if uncomplicated by secondary infection, and various other diseases.

6. With the method of preparation described in this paper, one has a most convenient and rapid means of making a general blood examination. From a single slide any observer can not only determine the presence or absence of iodophilia and of malarial parasites, but can estimate after a little practice, with surprising accuracy, the number of leucocytes. Any considerable change in the size and shape of the red corpuscles, and the presence or absence of nucleated forms, is plainly evident, while the various types of leucocytes are sufficiently differentiated to permit of a reasonably accurate differential count. The specimens will keep for weeks.

BIBLIOGRAPHY.

1. A. Czerny. Zur Kenntniss der glykogenen und amyloiden Entartung. Archiv für experimentelle Pathologie und Pharmakologie, 1893, Bd. xxxi.

2. Ehrlich. Ueber das Vorkommen von Glykogen im diabetischen und normalen Organismus. Zeitschrift für Klinische Medicin, 1882, Bd. vi.

3. G. Gabritschefsky. Microskopische Untersuchungen über Glykogenreaction im Blute. Archiv für experimentelle Pathologie und Pharmakologie, 1891, Bd. xxviii.

4. GOLDBERGER & WEISS. Die Jodreaction im Blute und ihre diagnostiche verwerthung in der Chirurgie. Weiner Klinische Wochenschrift, 1897.

5. HOFBAUER. Über das Vorkommen jodophiler Leukocyten bei Blutkrankheiten. Centralblatt für Innere Medicin, 1899.

6. HUPPERT. Ueber das Vorkommen von Glycogen im Blut und Eiter. Zeitschrift für physiologische Chemie, 1894, Bd. xvii.

7. KAMINER. Über die jodempfindliche Substanz im Leukocyten beim Puerperalfieber. Berliner klinische Wochenschrift, 1899.

8. ———. Leukocytose und Jodreaktion im Leukocyten. Deutsche Med. Wochenschrift, 1899.

9. LIVIERATO. Üntersuchungen über die Schwankungen des Glykogengehalten im Blute gesunder und kranker Individuen. Deutsches Archiv für klinische Medicin, Bd. 53, 1894.

DUNHAM. Iodophilia. Boston Medical and Surgical Journal, 1901.

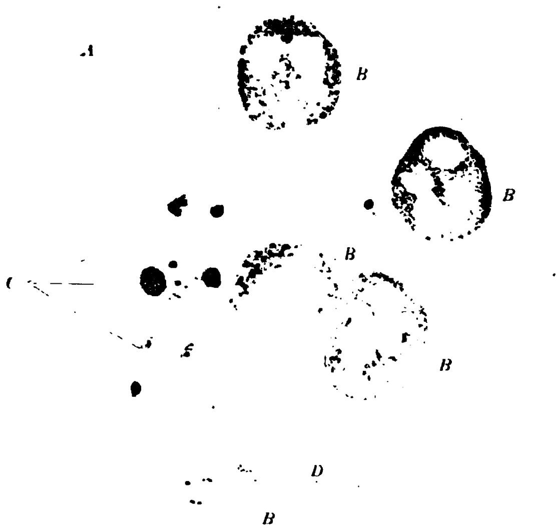
PLATE II.

(The figures were drawn with Zeiss, Oc. no. 2, Homogeneous Immersion, 1/12.)

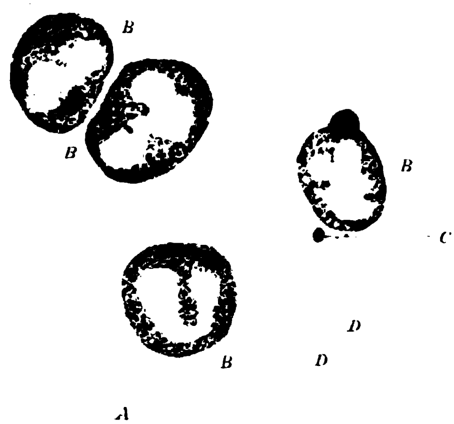
FIG. 1. — Case of general septicemia.
- *A*. Normal neutrophile.
- *B*. Neutrophile, showing reaction.
- *C*. Extra-cellular reaction.
- *D*. Red blood corpuscles.

FIG. 2. — Case of general peritonitis.
- *A*. Normal neutrophile.
- *B*. Neutrophile, showing reaction.
- *C*. Extra-cellular reaction.
- *D*. Red blood corpuscles.

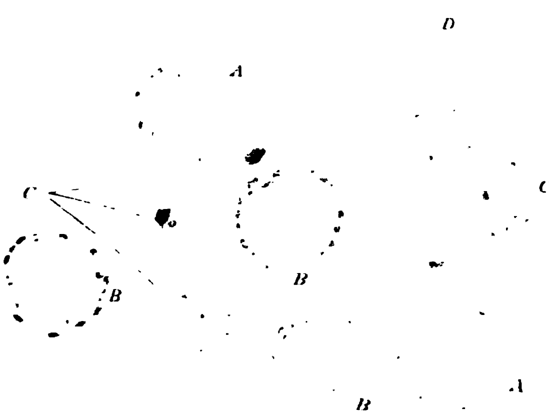
FIG. 3. Case of myelogenous leukemia.
- *A*. Neutrophile, showing reaction.
- *B*. Myelocytes, showing reaction.
- *C*. Extra-cellular reaction.
- *D*. Red blood corpuscles.



1



2



Locke & Cabot

3

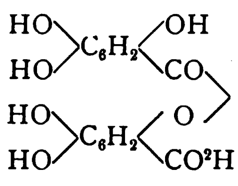
Iodophilia

COFFEE AND TEA AS PRECIPITANTS FOR POISONS.

TORALD SOLLMANN, M.D.
(*Assistant Professor of Pharmacology, Western Reserve University, Medical Department, Cleveland, O.*)

Various text-books mention strong tea or black coffee as chemical antidotes against alkaloids and metallic poisons (*e.g.*, Kobert, Pharmako-Therapie, 1897, p. 180). An investigation of the literature available to me failed to show any experimental support of these statements. The opinion of their value appears to be based solely upon clinical experience, and upon the fact that both beverages contain some form of tannin, gallotannic acid being known to precipitate both of the above classes of poisons. As to the clinical results, these might conceivably be based upon the physiological effects of the caffeine, rather than on the chemical action of the tannin, as is indeed acknowledged for the use of coffee in morphin-poisoning. That the chemical reactions of gallotannic acid should be supposed to hold true for the tannin of coffee and tea seemed to me unjustifiable, in the absence of direct experiments, since the different tannins are known to differ widely, not only in their composition, but also in their reactions.

The *ordinary tannin* — gallotannic acid — is an anhydride of digallic acid, having the composition $C_{14}H_{10}O_9$, and the structural formula[1]



It was formerly believed to be a glucosid, but this opinion was proven erroneous.

No recent statement could be found regarding the nature of the *tannin of tea*. Dragendorff[2] considers it for the

most part identical with gallotannic acid, Rochleder [10] with quercotannic acid, and it certainly agrees with these in most of its reactions. Stenhase,[3] however, believed it to be a different substance. The uncertainty is sufficient to make it questionable whether the reactions of one would hold for the other.

The tannic acid of coffee — *caffeotannic acid* — has been quite thoroughly investigated as regards its composition. It is a different substance altogether, being a diglycosyl ether of 3, 4 — cinnamic acid, having the composition $C_{21}H_{28}O_{14}$ * and the structural formula

$$C_6H_3\begin{cases} CH\ CH\ CO_2H \\ OC_6H_{11}O_5 \\ OC_6H_{11}O_5 \end{cases}$$

Very few statements could be found in the literature [(2)–(5)–(6)–(7–p. 901)] as to its reactions. Brühl states that it gives a greenish color to greenish-black precipitate with ferric chlorid ($Fe_2\,Cl_6$); and that it is precipitated by some metallic salts, but only from absolutely neutral solutions. Günther, quoted by Dragendorff,[2] states that glue, copper, and lead do not precipitate it quantitatively. The very markedly less astringency of coffee as compared with tea would suffice to indicate that the reactions of the tannins of the two substances are not identical, especially when it is noted that unroasted coffee contains, according to Spencer[7] (p. 902), five and eight-tenths to thirty-three and eight-tenths per cent of tannin, whilst tea, according to the same authority (pp. 887 to 895), contains only from four and eight-tenths to fifteen and four-tenths per cent, the latter being a very rare figure. Notwithstanding this smaller content in tannin, tea is in practice usually preferred to coffee as a chemical antidote.

The obscurity which obtains in regard to the chemical reactions of tea and coffee with the classes of alkaloids and metals is still greater if we inquire into the reactions with the individual members of the classes, the general statement of

* Beilstein [6] gives its composition as $C_{15}H_{18}O_8$.

text-books being that tannin precipitates " most alkaloids and metals." Dragendorff[8] gives a table of the reactions of gallotannic acid with certain alkaloids, but this lacks some important poisons. Bing[9] also mentions that morphin is not readily precipitated by tannin.

Most of the questions raised by the foregoing considerations were capable of solution by a few simple experiments, which I have carried out as follows:

A decoction of coffee was prepared by boiling for forty-five minutes ground, roasted coffee with ten parts of water, replacing from time to time the liquid lost by evaporation, filtering whilst hot, and percolating through the marc and filter enough hot water to make ten parts. A decoction of black " English Breakfast " tea was made in a precisely similar manner. Both liquids were somewhat acid to litmus. The coffee became somewhat turbid on cooling. The tea showed a very pronounced diffuse precipitate, and became almost opaque in thick layers. This could not be removed by filtration through paper. It could be made to disappear by heating or by the addition of alcohol. On account of this turbidity the reactions were always compared with corresponding dilutions of the decoctions with water. Neither decoction gave any precipitate with dilute hydrochloric acid, nor with Mayer's reagent, in the porportions which were used. The tests were made by adding definite proportions of the decoctions to solutions of the substances to be investigated, and noting the resulting phenomena at once, and after standing. If a turbidity or precipitate occurred, a portion of the unfiltered liquid, in the case of alkaloids, was mixed with about one-fifth volume of five per cent hydrochloric acid, and with one volume of alcohol, to test the solubility. Another portion of the liquid was filtered, and a part of the filtrate was put with more of the decoction. If no further precipitate occurred, a few drops of Mayer's reagent were added. In the case of metallic salts the decoction was added until a further portion ceased to affect the filtrate, and the latter was then tested for the metals.

The proportions usually employed for the alkaloids were two cubic centimeters of one to one hundred aqueous solution of the alkaloid * to one cubic centimeter of the decoction (expressed in the table as 1:150 — 3⅓ per cent.), or five cubic centimeters each of one to one thousand solution of alkaloid, and of the decoction (expressed as 1:2000 — 5 per cent.).

In the following I give the results arranged in groups, the least precipitation being found toward the head, the most toward the foot of the columns, and the reactions obtained by use with coffee and tea being placed parallel with the results of Dragendorff on gallotannic acid.

* Or one of its salts, brought into solution if necessary by the addition of a few drops of 5 per cent H_2SO_4.

A: ALKALOIDS.

TABLE OF PRECIPITATIBILITY OF ALKALOIDS BY COFFEE, TEA, AND TANNIN.

COFFEE. — Groups I. to IV. give no turbidity in 1:2000—5% (e).	**TEA.** — All alkaloids enumerated under Groups I. to IV. of coffee give immediately large precipitates with 1:150—$3\frac{1}{3}$% (b). The following statement refers to *mixtures of* 1:2000—5% (e).	**TANNIN.** — Results given by Dragendorff[8] for solutions containing 3:1000 of alkaloid.
GROUP I. No turbidity in 4:150—$3\frac{1}{3}$% (a) . . . . . *Morphin.*	GROUP I. Very slight turbidity; 1:1000—5% (f) gives marked precipitation, *Atropin.*	GROUP I. Slight turbidity, disappearing immediately on adding cold HCl, *Morphin, Caffein.*
GROUP II. No turbidity in 1:150—$3\frac{1}{3}$% (b) . . . *Atropin, Cocain, Coniin, Pilocarpin, Pyridin, Spartein, Brucin?* (c).	GROUP II. Very small, diffuse precipitation, *Pyridin, Coniin.*	GROUP II. Turbidity, increased by cold HCl, but dissolving in warm HCl, to reappear on cooling, *Narcotin, Aconitin.*
GROUP III. Faint but undoubted turbidity in 1:150—$3\frac{1}{3}$% (b), *Aconitin* (d), *Nicotin.*	GROUP III. More abundant, diffuse precipitation, *Morphin.*	GROUP III. Small precipitate, behaving as Group II. . *Veratrin.*
GROUP IV. Marked turbidity in 1:150—$3\frac{1}{3}$% (b) . . . . *Lobelin.*	GROUP IV. Abundant diffuse precipitation; on standing visible collection of powdery precipitate on bottom of tube, *Cocain, Pilocarpin, Brucin, Aconitin, Lobelin, Nicotin, Veratrin.* These four groups filter very turbid.	GROUP IV. Abundant precipitate, soluble in cold HCl, *Strychnin, Brucin, Codein, Atropin, Physostigmin.*
GROUP V. Precipitates in 1:2000—5% (e): *Veratrin* (g). — Very small precipitate. *Strychnin.* — Considerable precipitate. *Quinin.* — Large precipitate. *Hydrastinin, Cinchonidin, Cinchonin.* — Very large precipitate. *Apomorphin.* — Largest precipitate.	GROUP V. Immediate curdy precipitate; supernatant liquid quite clear and lighter in color. *Spartein:* Smallest precipitate, liquid of deepest color. *Strychnin:* *Quinin:* *Hydrastinin:* *Cinchonidin:* *Cinchonin:* *Apomorphin:* Largest precipitate, liquid of lighter color.	GROUP V. Abundant precipitates, soluble only in warm HCl, *Quinin, Quinidin, Cinchonin, Conicin, Nicotin.*

(a) 2 cc. of 4% solution and 1 cc. of decoction. (b) 2 cc. of 1% solution and 1 cc. of decoction. (c) Doubtful turbidity. (d) German amorphous. (e) 5 cc. of .1% solution and 5 cc. of decoction. (f) 5 cc. 1:500 solution and 5 cc. of decoction. (g) Schuchardt; probably mixed alkaloids.

Character of the precipitates. — The reaction occurs immediately in all cases. When the precipitate is small, it shows as a fine turbidity; if somewhat larger, a fine powder collects in time at the bottom of the tube. If still larger, the precipitate is flocculent to curdlike. It carries with it a large amount of the coloring matter of the decoctions, the latter becoming lighter in color and clearer in proportion to the size of the precipitates. These are colored brown. (When produced by pure gallotannic acid, they are white or yellowish.)

Solubility of the precipitates. — The precipitates, by whatever tannin produced, are *soluble in fifty per cent alcohol.* In regard to *solubility* in *hydrochloric acid*, the general statement is usually made that the tannates of alkaloids are soluble in dilute hydrochloric acid. From the results of Dragendroff, quoted in the table, it will be seen that the gallotannates of Atropin, Brucin, Caffein, Narcotin, Nicotin, Quinidin, Quinin, and Veratrin dissolve only on heating. With coffee I found that the addition of hydrochloric acid to about one per cent caused complete solution, in the cold, only in the case of Pyridin. Partial solutions occurred, however, in all cases, the mixture becoming clearer, the precipitate less, and the filtrate from this giving stronger alkaloidal reactions than when the same amount of acid is added to the previously filtered liquid.

Amount of the decoctions necessary to complete the precipitation. — I did not attempt to determine the minimum amount of the decoctions which would complete the precipitation, but contented myself with observing whether or not the addition of a further amount of the decoctions produced any change in the filtered liquids. There was no change in all the cases in which the original mixture showed only turbidity, or a small precipitation. This disposes of the Groups I. to IV. in mixtures of one to one hundred and fifty minus three and one-half per cent for coffee, and one to two thousand minus five per cent for

tea. Group V. of coffee also showed no further precipitation, so that one cubic centimeter of a ten per cent decoction of coffee is more than enough to complete the precipitation of one milligram of any of the precipitable alkaloids which were tested.

In the experiments with tea, the decoction caused further precipitation in all the mixtures of one to one hundred and fifty minus three and one-half per cent which were tried (Atropin, Cocain, Morphin, Pyridin, and Spartein); so that one-twentieth cubic centimeter of a ten per cent decoction per milligram of alkaloid was in no case sufficient to complete the precipitation. In the proportions of one cubic centimeter of a ten per cent decoction per milligram of alkaloid, precipitation was complete for Apomorphin, almost complete for Quinin and Strychnin, not at all complete for Cinchonidin and Hydrastinin. Three cubic centimeters of a ten per cent decoction per milligram completed the precipitation in every case.

Completeness of the precipitations. — The presence of alkaloid remaining in solution after the precipitation by the decoction was completed, was tried for by the addition of Mayer's reagent. This trial was of course superfluous when the decoctions had produced no precipitates.

The filtrates from solutions of one to one thousand, completely precipitated with the decoctions, gave with Mayer's reagent:

FOR COFFEE.

No turbidity: Apomorphin and Veratrin.

Turbidity only: Quinin.

Large precipitate: Cinchonidin, Cinchonin, Hydrastinin, Strychnin.

FOR TEA.

No turbidity.	Turbidity only.	Small precipitate.	Large Precipitate.
	Smallest:		Smallest:
Apomorphin.[1]	Atrophin.	Veratrin.	Hydrastinin.
	Morphin.	Quinin.	Strychnin.
	Pyridin.	Cinchonidin.	
	Spartein.	Cinchonin.	
	Brucin.	Cocain.	
	Coniin.	Pilocarpin.	
	Aconitin.		
	Lobelin.		
	Largest.		Largest.

The precipitation is therefore only complete with apomorphin.

PRECIPITATION OF METALLIC SALTS.

We have already quoted above the statement of Brühl that coffeo-tannic acid precipitates some metals from strictly neutral solution. The United States Dispensatory (18th ed., p. 101) says that it precipitates the salts of Ferric oxid, Lead, Silver, Copper, Uranium, Chromium, Mercury, Antimonous Oxid, and Stannous Oxid.

Directing my attention particularly to those metals which are of toxicologic importance, I found that coffee and tea give very similar results, although the precipitation by the latter is more complete. The precipitates occur at once as a turbidity which soon collects into flakes or a curd-like coagulum. They are highly colored, the liquid becoming correspondingly lighter and clearer. Hydrochloric acid in the proportion of one per cent. does not cause them to disappear.

[1] A 1–2000 solution of apomorphin gives a very distinct precipitate with Mayer's reagent, so that the want of this reaction shows that the alkaloid has been completely precipitated by the decoction.

GROUP I. — No precipitation: *Arsenious Acid and Tartar Emetic:* The former leaves both liquids quite unaffected. The Antimony and Potassium Tartrate causes no change in the coffee, but produces a turbidity in the tea. This cannot be removed by filtration. The addition of Hydrochloric acid causes it to go into brown flakes. The filtrate from this contains much Antimony, as shown by orange precipitate with Sulphuretted Hydrogen.

GROUP II. — Partial precipitation: The decoctions cause a large precipitate, but the filtrate from this gives a precipitate with ferrocyanide when it precipitates no further with an additional portion of the decoctions: *Cobalt chlorid; Cupric sulphate; Nickel sulphate; Uranium acetate; Zinc sulphate.*

Ferric chlorid causes a greenish-black precipitate, which cannot be removed by filtration.

GROUP III. — Complete, or practically complete, precipitation: The filtrate from the abundant precipitates shows no or very faint reactions of the metal.

Lead nitrate: The filtrate is unchanged by Hydrochloric acid, but gives a faint turbidity, with H_2SO_4.

Silver nitrate: Sodic chlorid causes scarcely any turbidity with the filtrate from tea; some turbidity, but no precipitate, with that from coffee.

Aluminum chlorid: The filtrate remains clear on adding $NH_4Cl + NaOH$.

GROUP IV. — *Mercuric chlorid* deserves to be placed in a special group. It is not affected in the least by coffee, but a turbidity is produced by tea, the filtrate giving some reaction with ammonium sulphid.

PROTEIDS.

The differences in the reactions of tea and coffee, which the investigation brought to light, made it appear interesting to inquire also into their reactions with a few proteids. Egg-white, albumose, and gelatine were tried.

	Gives on addition of:	
	Equal volumes of 10% coffee decoctions.	Equal volumes of 10% tea decoction.
A solution containing 20% of moist *white of egg*	A slight turbidity at once, but no precipitate	Abundant precipitate.
A 2 % solution of *gelatin*, heated to liquefaction:	No change	" " " "
A 10 % solution of peptone-(Witte):	" " "	" " " "

The decoctions, boiled with one-quarter volume of the egg solution and filtered, still contain sufficient tannin to precipitate strychnin from 1:1000 solution.

CONCLUSIONS.

I. PRECIPITATION OF ALKALOIDS. — Atropin, coniin, morphin, and pyridin are not precipitated even in fairly strong solution by coffee. Tea precipitates them from strong, but not from weak, solutions.

Aconitin, brucin, cocain, lobelin, nicotin, and pilocarpin in weak solution are only sparingly precipitated by tea; coffee does not affect them even when they are in concentrated solutions.

Apomorphin, the cinchona alkaloids, hydrastinin, strychnin, and veratrin in dilute solutions are precipitated efficiently by either coffee or tea, the latter being generally more efficient, except perhaps for veratrin and quinin.

The precipitation is incomplete with all alkaloids except apomorphin. However, the quantity of unprecipitable alkaloid is quite small in those which are said to precipitate from "dilute solution," since most of the alkaloid is removed from 1 : 2000 solutions.

The precipitates are somewhat soluble in dilute HCl, very readily soluble in dilute alcohol. The administration of the latter must therefore be avoided if these beverages (or tannin)

are used as chemical antidotes in alkaloidal poisoning. Since the precipitates are not quite insoluble in water, as little liquid as possible should be given. The quantities of the decoctions should not be less than three cubic centimeters of a well-boiled ten per cent decoction for each milligram of alkaloid.

II. METALLIC SALTS. — Tea is also the more efficient precipitant of metals, but the difference is not nearly so striking as with alkaloids. Both beverages are inefficient against arsenious acid or tartar emetic. They precipitate to a large extent, but not quite completely, the salts of cobalt, copper, nickel, uranium, and zinc, and would be useful antidotes against the toxic members of this list. They precipitate practically completely the salts of aluminum, lead, and silver. Mercury is partly precipitated by tea, but not by coffee, so that the former would be an antidote, the latter not.

III. PROTEIDS. — These differentiate very sharply between the two tannins: whereas tea produces large precipitates, coffee leaves them unaffected, or renders them slightly turbid at most. This serves to explain the less astringent taste of coffee and its less deleterious effect upon digestion.

The reactions of tea bear a very close resemblance to those of gallotannic and quercotannic acid. The precipitant effects of caffeo-tannic acid are weaker, but occur along the same lines. The greatest differences are seen in their action on proteids and on certain alkaloids, whereas other alkaloids and most metallic salts are precipitated almost equally well by both. An exception is formed by mercuric chlorid, which is partly precipitated by tea, not at all by coffee.

BIBLIOGRAPHY.

1. Roscoe and Schorlemmer. Ausf. Lehrb. d. Chem., 1886, Bd. 4, II., s. 656.

2. Dragendorff. Pflanzanalyse, 1882, s. 166.

3. Stenhase: quoted by U.S. Dispensatory, 18th ed., p. 1810, from Am. J. Pharmacy, 1862, p. 254.

4. Kunz-Krause. Ber. deutsch. chem. Ges. 30, II., s. 1617, 1897; quoted by

5. Brühl, in Roscoe and Schorlemmer, Bd. 8, s. 664, 1901.

6. Beilstein. Organ. Chem., Bd. 2, s. 2071, 1896.

7. G. L. Spencer. "Tea, Coffee, and Cocoa Preparations," U.S. Dept. Agric., Bull. 13, 1892.

8. Dragendorff. Toxicologie, Ed. Francaise, 1886, p. 179.

9. Binz. Pharmakologie, 1891, s. 356.

10. Rochleder: quoted in Beilstein, 1897, Vol. 3, p. 688, from Liebig's Anal., Vol. 63, p. 205.

DERMOID CYSTS AND TERATOMATA OF THE ANTERIOR MEDIASTINUM.

HENRY A. CHRISTIAN, A.M., M.D.
(*From the Pathological Laboratory of the Boston City Hospital.*)

The general subject of dermoid cysts and teratomata is one of much interest. This is due in part to their relative infrequency; in part to the problem of their genesis and the many theories which have been advanced for its solution. In regions of the body, as the ovary, they are comparatively common; in others they are quite unusual. In the anterior mediastinum they are so infrequent as to seem to justify the report of a new case, the attempt to draw some conclusions from the cases previously reported, and some discussion as to their origin in this particular region.

The patient whose case we have to report, is one of Drs. McAllister and Davis, of Lawrence, Mass., from whom the following brief history was obtained:

Patient is a female, age thirty-eight years. She is married and has had one child. For the past six years she has complained of what she termed trouble in the chest, and gives a history of night sweats. For two years she has coughed up hairs at intervals. These have generally been few in number. For five weeks she has suffered from severe pain in the chest and has had cough with purulent expectoration. During this time there have been several attacks of hæmoptysis, during some of which she has almost strangled. On physical examination there is dulness in the right chest, and this has been noted to decrease in extent after attacks of coughing, during which much material was brought up.

Several hours before death, which occurred March 21, 1901, there was a gush of blood and pus from the mouth and with this material were mixed a few hairs.

At autopsy (B.C.H. — U. '01.8) a tumor as large as a cocoanut is seen occupying the lower portion of anterior mediasti-

num. On opening this a pint of fluid and solid material escapes. Lungs and heart removed *en masse.*

Left Lung. — Pleural and diaphragmatic surfaces smooth and glistening. Anterior part of lower border of upper lobe is adherent to adjacent pleuro-pericardium by fibrous bands which are easily broken up. Lung appears normal.

Right Lung. — Surface is covered with shreds of old fibrous adhesions which everywhere bind lung to parietes. Between lower and middle lobes there are slight adhesions; between upper and middle lobes the division is obliterated. The middle lobe is small and only the lower portion comes to the surface, the other parts being hidden beneath the upper lobe. The entire lung is pushed backward and to the right by the tumor mass. The right lung is slightly increased in density and is distinctly less voluminous than the left, but there are no firm areas to be felt except in immediate connection with the tumor wall.

Bronchi are congested. Their mucous membrane is smooth and glistening, apparently not thickened. Little secretion.

Peribronchial Lymph Nodes deeply pigmented, not enlarged.

Pericardium. — Parietal layer in many places is covered with villous masses of fibrin. These are most abundant on surface adjacent to the tumor. In other places surface is roughened in ridges, but is at the same time glistening. There are scattered everywhere minute sub-pericardial hemorrhages. This condition is particularly marked on surface adjacent to the tumor. Visceral layer, except over lower portion of left ventricle, shows similar, larger, longer, and more profuse villous masses of fibrin which are pulled off and torn apart with ease. Surface of lower portion of left ventricle is glistening, is slightly roughened by thickenings of pericardium, and shows numerous small subpericardial hemorrhages.

Tumor. — Between the pericardium and the inner surface of the right lung there is a large tumor mass. This extends from the diaphragm to a point 4.5 cm. above the bifurcation of the trachea and is attached to the whole inner sur-

face of the right lung from its base to a point 6.5 cm. below its apex. The upper limit of the tumor mass is approximately on a level with the top of the arch of the aorta. It is in apposition to the diaphragm over an area about 9 cm. in diameter, but not adherent to it. Anteriorly it is bounded by the chest wall and a small part of the lower portion of the upper lobe of the right lung. Posteriorly it is in direct relation with the lower lobe and lower part of upper lobe of right lung. The right primary bronchus lies just behind and in close relation to the upper portion of tumor mass.

There is no pedicle to tumor and no process connecting it with organs of neck or structures forming anterior chest wall. A vertical incision, 14 cm. long, in the front of the tumor has allowed the escape of considerable fluid and solid material. There remains in the cavity about 200 cc. of white material, in places slightly tinged with yellow. This is greasy, soft, friable, and full of long, delicate, curly, blond hairs. These hairs are almost white, and approximate one-half the diameter of the ordinary fine human hair. Some are 4 to 5 cm. long. Many are tapering at one end.

The cavity distended measures about 14 by 12 by 10 cm.; its longest diameters are the vertical and antero-posterior. The anterior and lower wall of cavity is of firm, dense white fibrous tissue, 2 to 3 mm. in thickness. Over the anterior part between wall of tumor and pleura there is in places a layer of fat and fibrous tissue, 5–6 mm. thick. In the lung tissue adjacent to tumor wall there is evidently a considerable fibrous tissue increase which causes an apparent thickening of the wall on that side.

The cyst is lined with a smooth, glistening, whitish membrane in which are a few fine congested blood vessels. In the right half of the tumor there are small, scattered, bony plates adherent to the wall. The entire inner surface of tumor is trabeculated, and running out from the main cavity are several small cul-de-sacs, varying from 0.5 to 1.5 cm. in greatest diameter. Between one of these and a medium-sized bronchus there is an opening 0.5 cm. in diameter. This opening lies just at the beginning of the upper branch

of the main bronchus. Other bronchi are in very close relation to the cavity, but do not open into it.

In parts of the tumor the trabeculæ are very well developed. Many of them are distinct rounded cords, 1 to 3 mm. in diameter and 4 to 10 cm. in length, running from one side to the other of cyst cavity. They are of firm fibrous tissue. Across the upper part of the cavity runs a trabecula 6 cm. long. Attached to this on one side is a mass shaped like the segment of an orange which is 4 cm. long, 2 cm. broad, and 2 cm. thick at its outer edge. On the other side is an irregular spoon-shaped tag, 4 cm. long, 3 cm. broad, and 0.5 cm. thick. Near this is another band of fibrous tissue 5 cm. long. Attached to it is a mass 5 cm. long, 4 cm. broad, and 1.3 cm. thick. From these the hair seems to have had its origin, because long hairs are attached here.

Microscopic Examination. — (Technique — Zenker's fluid — paraffin — eosin and alkaline methylene blue.)

Sections through the polypoid excresence show an irregularly undulating surface covered with epidermis. Externally there is a distinct stratum corneum. Beneath this there are a few flattened cells, some of which show eleidin granules. These pass by gradual transition into large polygonal prickle cells forming a layer five or six cells deep. Next to the corium is a somewhat poorly defined layer of cuboidal cells forming an indistinct stratum Malpighii. Papilla formation is almost entirely absent, though there are at points slight epithelial downgrowths into the subepidermal tissue. In this tissue are numerous well-developed sebaceous glands and a very few hair follicles out of which the hairs have fallen, but no sweat glands. The subepidermal tissue consists of loosely woven connective tissue with scattered foci made up of a moderate number of plasma cells, a few lymphoid cells, and an occasional polymorphonuclear leucocyte. In the central part is a small amount of adipose tissue. A fair number of blood-vessels is present.

Sections through the cyst wall show on the surface a similar layer of epidermis with a slight stratum corneum and no papilla formation. No hairs, sebaceous glands, or sweat

glands are present. The epidermis lies directly on a dense, cell-poor, very slightly vascular layer of lamellated, somewhat hyaline connective tissue. Beneath this is a layer of adipose and connective tissue which is more vascular and contains numerous lymphoid and plasma cells.

In five hundred and twenty cases of mediastinal disease, exclusive of diseases of the heart and aorta, collected by Hare[1] in 1889, there are two hundred and eighty-eight cases of true tumor. Of these ten are dermoid cysts. (Hare tabulates eleven cases of dermoid cyst, but his ninth, attributed to Naumann,[2] is the same case as his first reported by Lebert.[3] His eleventh case attributed to Finkler[4] is more usually given as Pinders'[5] Case I). Hoffmann[6] (1896) collected twelve cases of dermoid cyst of the anterior mediastinum. Planz[7] reported twenty-five, but of these the case of Spath[8] does not seem justly included, since it appears to be a malignant tumor or possibly a tubercular process originating from the vertebræ. At any rate there is no evidence of the presence of cysts of a dermoid character, and the author himself did not regard it as being of this nature. Finally Ekehorn[9] in 1898 gathered twenty-nine and added two cases of his own. To these we have been able to add several from the literature, and with our own have tabulated forty cases. Though this list is possibly not complete, it comprises the vast majority of reported cases since the first of Gordon,[10] whose case was presented Nov. 25, 1823, though his paper was not published until 1827.

Sex. — If we analyze these cases, we see that the sexes are fairly evenly represented. Of those in which the sex is stated seventeen were males, fourteen females.

Age. — That a very large proportion occur in young adult life is very strikingly shown.

10 to 20	years,	1	case.
20 " 30	"	19	cases.
30 " 40	"	7	"
40 " 50	"	2	"
Over 50	"	2	"

Dividing a little differently:

Under 20	years,	1	case.
20 to 35	years,	21	cases.
35 " 50	"	5	"
Over 50	"	2	"

These figures refer to the age at the time of death or operation. If we recall that several cases showed symptoms or physical signs a number of years before this time, then the preponderance of those occurring in young adults becomes more marked.

Symptoms. — In seven cases no clinical history is given. In six more it is evident that the previous symptoms had no connection with the existent tumor. In these cases death was due to other causes and the tumor in the anterior mediastinum was of the nature of an accidental post-mortem finding. Where the tumor did produce symptoms these were most frequently referable to the respiratory tract. In fifteen cases there was more or less dyspnea or evidence of pressure on the trachea or bronchus. In sixteen cases there was cough, and in twelve of these free expectoration, often with foul odor. In seven hemoptysis occurred. What is most interesting was the coughing up of hair which occurred in eight cases, those of Mohr, Cloetta, Kückmann, Godlee, Sormani, Nobiling, Ekehorn's second case, and our own. This occurred at various stages of the disease, in Mohr's case fourteen and in Sormani's sixteen years before death.

In only one case is there a history of difficulty in swallowing. Pain in the chest is not a prominent symptom and is noted in only five cases. Only twice was there local edema or other evidence of involvement of the blood-vessels, and in these two cases malignant growth was associated with the benign.

Physical Signs. — In eleven cases no note of physical examination is given. In six the chest on the side of the tumor distinctly bulged. In four cases the tumor appeared in the neck through the superior aperture of the thorax. In four

Number	Reported by	Sex.	Age	Symptoms	Duration	Physical signs.	TUMOR Position	Size	Structure
1	Gordon [10]	F.	21	Symptoms of pressure on respiratory tract.	1½ yrs.	Pulsating tumor in neck.	Behind upper ⅓ of sternum.		Cyst with fatty debris, hairs, bone and teeth
2	Mohr [11] also Munz [12]	F	28	Cough Hair in sputum	14 yrs.	Diminished expansion left chest Dulness Respiratory sounds absent.	Left lung communicates with a bronchus	Large	Cyst lined by epidermis with hair follicles, sebaceous and sweat glands, cartilage and bone, greasy contents with hairs
3	Buchner [13]	F.	36	Pains Expectoration Hemoptysis Rapid respiration	½	Dome-shaped projection of right chest, 2nd to 4th rib. Dulness	Right chest, 2nd rib to diaphragm.	Child's head	Cyst with fatty debris, hairs, cholesterin, cartilage and bone.
4	Lebert. [3]	M.	60	Dyspnea cough, bloody sputum	Many years	Behind upper ⅔ of sternum			Multilocular cysts lined by squamous or cylindrical epithelium Excrescences Cartilage
5	Cordes [14]	M.	28	No definite symptoms		Heart dulness much increased and sounds weak	Behind upper ⅔ of sternum	Man's fist	Cyst partly lined by epidermis with hair follicles and sebaceous glands, hair & cartilage
6	Cloetta [15]	F	20	Symptoms of chronic pulmonary tuberculosis Hair in sputum			Lower left lobe of lung and mediastinum	Apple	Cyst with hair and greasy material Excrescences, cartilage and bone
7	Salomonsen [16]	F.	24	Cough, pain, expectoration, hemoptysis.	10	Dulness with weakened breath sounds.	Close to hilum of right lung	Pigeon's egg	Cyst lined by epidermis with hair follicles and sebaceous glands Bone
8	Collenberg [17] also Waldeyer [18]	M.	44	No history obtained		Cyanosis	Right thorax Sixth rib to diaphragm Pedicle joining thyroid.	Larger than child's head.	Calcified cyst lined by epidermis with sebaceous glands Contents hair and greasy material
9	Nebiling [19]			Hair in sputum			Lungs.		Dermoid cyst
10	Villard [20]	F	25				Between right lung and heart		Cyst with some cornified epithelium Contents greasy material and no hair
11	Pöhn [21]	M	34		2+	Fluctuating tumor in neck	Neck and upper thorax		Cyst containing fatty material and hair
12	Virchow [22]	M	22	Pain, shortness of breath		Dulness Breath sounds absent. Painful nodule in chest wall with edematous swelling	Right thorax	20x21x13 cm	Partly multilocular cyst lined by epidermis Contents brei and hair Partly solid of striated muscle cartilage, glands, carcinomatous & sarcomatous nature
13	Kuchmann [23]	M	33	Shortness of breath Hairs in sputum	2	Fluctuant tumor in neck.	Neck and upper thorax		Cyst with puriform material and hairs
14	Gäser [24]								Cyst with hair
15	Marchand [25]	F	27				Right thorax 2nd to 5th rib Process to thyroid	Child's head	Cyst lined by epidermis with hair follicles and glands. Contents fatty brei and hair Thymus corpuscles.
16	Paget. [26]						Behind manubrium sterni		Cyst with hairs and sebaceous glands
17	Pinders I. [1] also Finkler & Ribbert [27]						Ant. mediastinum 1st to 5th rib	Greatest dia. 17 cm.	Cyst with fatty caseous contents Lymphosarcoma
18	Pinders II [1]	M.	53				Near pericardium and left main bronchus	Goose egg.	Cyst with grayish yellow brei.
19	Loewenmeyer [28]	M	.	Hemoptysis Shortness of breath	4	Dulness Bulging of left chest. 1st to 6th rib	Left thorax.	Child's head	Cyst lined by epidermis with fatty material and hair. Minor cysts lined by ciliated epithelium Cartilage
20	Sangalli. [29]	F	30	Long suffering			Right thorax, middle lobe to diaphragm	28x16 cm	Multilocular cyst lined by epidermis Atheromatous contents with hairs Cartilage and bone.
21	Godlee. [30]	F	29	Pain, shortness of breath Hair coughed up	8	Dulness, 3rd rib to liver Breath sounds absent.	Right thorax.	Large	Cyst with skin-like wall Excrescences
22	Sormani [31].	F.	26	Cough. Hair in sputum Dyspnea Pain	16		Left lung	Child's head	Cyst with skin-like wall. Contents atheromatous brei and hair.
23	Marfan. [32]	M.	30				Ant. mediastinum, base of neck to pericardium	Fist	Cyst, no epithelium Contents fatty material and hair
24	Koerte [33].	M.		Pressure symptoms		Fistula in anterior chest wall	Behind upper sternum.	Fist	Cyst lined by epidermis Hair Excrescences
25	Harris [34]						Between heart and left lung		Dermoid cyst Cartilage Bone
26	Joves [35]						Left thorax	Large	Cysts lined by epidermis with hair follicles and sebaceous glands or with cylindrical epithelium Teeth Cartilage. Sarcoma.
27	Bastianelli [36]	F	20		4	Fistula at base of neck	Neck and upper thorax	Nut	Cyst containing fat and hair
28	Dardignac [37] also Belin [38]	M	22	Difficulty in breathing	4	Projection of right chest 4th to 6th ribs Dulness	Right thorax	Large	Cyst containing hairs
29	Pflanz [7]	M	21	Difficulty in swallowing	1	Projection of upper right thorax. Dulness Breath sounds absent Fluctuant tumor in neck	Neck and thorax	Large	Cyst containing hair, flakes of epidermis and fatty debris
30	Smythe [39]			Difficulty in respiration Cough	3		Left thorax, 1st to 6th rib	Large.	Cyst lined by epidermis with hairs Bone
31	Jones [40]	M	9				Neck and thorax		
32	White [41]						Between pericardium and right lung	Tangerine orange	Cyst containing hair, and greasy material
33	Kretz [42]	M	30				Upper lobe of left lung extending into ant. mediastinum	Apple	Cyst lined by epidermis with empty hair follicles and sebaceous glands Excrescences Hair and bone.
34	Ogle [43]	M.	28	Cough Hemoptysis	5		Lower lobe of right lung extending into ant. mediastinum	6½ inches long	Cyst lined with skin Excrescences Hair. Tooth
35	Ekehorn I. [9]	F.	21	Cough Expectoration Hemoptysis	3+	Dulness. Weakened breath sounds	Right thorax and lower ⅔ of ant. mediastinum		Cysts lined by epidermis Hair. Teeth. Bone and cartilage Nerves and ganglia Parts suggesting respiratory & digestive Tracts. Choroid.
36	Ekehorn II. [9]	M	22	Spat up excretion containing hair	1			Fist. 7 cm.	Cysts lined by epidermis cylindrical and cuboidal epithelium Hair, cartilage and bone
37	Borgmann [44]	M	38			Swelling over sternum and adjacent 2nd and 3rd ribs Fistula	Superficial to sternum and communicating with intra thoracic cyst	Goose egg	Cyst lined by skin. Excrescences Hair. Teeth
38	Mandlebaum [45]	F	38			Fluctuant tumor over right 3rd rib	Between right lung and pericardium	8x6 cm	Cyst lined by epidermis Hair Thyroid-like vesicles
39	Sited by Hare [46]	No data obtainable							
40	Christian	F.	38	Cough Hemoptysis Hair in sputum. Pain	2+	Dulness.	Between right lung and pericardium, arch of aorta to diaphragm.	14x12x10 cm	Cyst lined by epidermis with hair follicles and sebaceous glands. Excrescences Hair Bone

cases fistulæ between the cyst and the cutaneous surface were formed. In ten there was dulness on percussion over the area occupied by the tumor. Auscultation in these regions showed respiratory sounds suppressed in four cases and greatly weakened in two more. Three cases gave signs of chronic pulmonary tuberculosis.

Duration. — This in most cases is very difficult to estimate. In twenty-three cases the onset of some symptom was definitely enough stated to form the basis of an estimation, and the figures given are approximately correct.

Duration of less than 1 year, 2 cases.
" " 1 to 2 years, 6 cases.
" " 2 " 3 " 6 "
" " 5 " 10 " 3 "
" " 10 " 15 " 4 "
" " more than 15 years, 2 cases.

Thus in the majority of cases reported the course of the disease has been a relatively slow one, a period rather of years than of months. In strong contrast to these are the cases of malignant disease of the same region reported by Harris,[47] in which the average duration is given as six months.

Position of Tumor. — In eighteen cases it was situated in the upper half of the thorax and was wholly or in part immediately behind the upper portion of the sternum. In eight it was situated in the lower half of the thorax between the heart and adjacent lung. In six cases it occupied nearly the entire pleural cavity. In one it had a position near the hilum of the lung. In six cases the tumor appeared in the neck in the superasternal notch, or above one sterno-clavicular articulation. In four the tumor was embedded in lung tissue.

The most frequent position, then, is the upper part of the anterior mediastinum. Here there is a potential space, between the sternum in front and great vessels behind, capable of accommodating a medium-sized tumor. Development of the tumor beyond a certain point in this region

would be resisted in front by the bony wall of the thorax, behind by the great vessels and trachea supported by the vertebral column. Growth would be possible in three directions: upward through the superior aperture of the thorax, laterally into the pleural cavity, and downward between the heart and lungs. These three directions of expansion are illustrated in different instances.

For some cases a previous position in the upper anterior mediastinum can be assumed. Portions of some of the largest tumors still extend into this region, and it would seem probable that with them a change of position has taken place along the lines of least resistance merely to accommodate their bulk adequately. In other cases there is proof of their development in this region and subsequent migration. In these we find pedicles extending from the tumor to the root of the neck (cases of Collenberg & Pinders). Lastly, the presence of structures in the tumor whose origin is referable to organs in this region, as in the case of Mandlebaum, supports this view. In several instances, especially those studied only at the time of operation, the description is too incomplete to be able to accurately localize the tumor or to form an opinion of its probable origin. In other tumors which were situated at a lower level and which were accurately studied there is no evidence of a different earlier position. Though for these there is some probability of the same origin from the upper anterior mediastinum, a primary development in the lower mediastinum cannot be excluded, a point which will recur in the discussion of the genesis of this group of tumors.

In four cases the tumor was in the lung. However, it does not seem probable that these originated in the lung, since the lung, entodermal in origin, could furnish no basis for these ektodermal structures. They have rather grown into the lung, most probably from the mediastinum, a theory supported by the gradations found between complete envelopment by lung tissues and simple adhesion to the surface of the lungs.

Relations to Other Parts. — In almost every case there

were adhesions to some adjacent organ, and often these were very extensive. The tumor was most frequently bound to some part of the lung, and almost as often to the pericardium. Less frequently they were attached to other structures, as chest wall, diaphragm, or great vessels. These adhesions would form a serious difficulty in any attempt at complete removal of the tumor, and in most cases would prevent anything further than incision and drainage.

These tumors, though occupying a position surrounded by vital organs and generally in actual union with them, do not as a rule produce great destruction of adjacent structures. In one case the aorta was eroded; in a second there was a communication between tumor and pericardial cavity. Much more frequently have they eroded into the lung and formed a communication with the bronchus. This happened in thirteen instances.

Size varies from a pigeon's egg to a tumor larger than a child's head. The larger tumors are more frequent, as might be expected, since the symptoms result from pressure, and these, as a rule, do not appear early in tumors which grow slowly and allow neighboring organs to accustom themselves gradually to changed conditions.

Structure. — A consideration of the structure of these tumors shows that they may be divided into three classes: 1st, those of slight complexity, which are essentially dermoid cysts of ektodermal origin; 2d, those of great complexity, which contain derivatives from all three germinal layers with the formation of rudimentary organs, and which may be regarded as teratomata; 3d, tumors of the first or second class which in some part of their structure are malignant and form metastases in other organs.

The first class constitutes a very large proportion of the reported cases. They occur as simple unilocular cysts, as unilocular cysts with diverticula of varying size, and as multilocular cysts. The cysts are either smooth or contain polypoid excrescences of greater or less complexity. Their walls are lined by epidermis containing, as a rule, hair follicles and sebaceous glands. Less frequently sweat ducts are

found. In one case the cyst showed no epidermis, but contained hairs, some of which were still embedded in the wall so that it was considered that the epidermis was formerly present, but had died and desquamated.

In four cases the cyst wall consisted simply of connective tissue. In eleven cases bony plates were present in the wall and bits of cartilage in an equal number. Both bone and cartilage were found in seven. In five cases there were teeth either free in the cavity or embedded in the wall. In some cases there was a distinct alveolar border.

Of slightly greater complexity of structure are a few cases. That of Lœwenmyer, besides a large cyst lined with epidermis, contained smaller ones lined with ciliated epithelium. The cases of Marchand and Pinders showed parts resembling thymus. In Mandlebaum's case there were vesicles identical with those found in the thyroid. In others there were cysts wholly or in part lined by cubical or cylindrical epithelium. These latter might be explained as a persistence of the stratum Malpighii of the epidermis with desquamation of the other layers.

Of the second class there have been only two cases, those of Ekehorn (Case I.) and Virchow. In Ekehorn's, Case I., besides a dermoid cyst, there was a bone resembling the superior maxilla, ganglia suggesting spinal ganglia arranged between bits of bone, and structures somewhat resembling fetal lung and intestine. Virchow's case showed much young striated muscle, and in places suggested fetal lung in addition to areas of a sarcomatous and carcinomatous nature.

The third class is represented by three cases, those of Pinders (Case I.), Jores, and Virchow. Pinders considered his case in part lympho-sarcoma. That of Jores was in places spindle-cell sarcoma and had metastasized in the right lung. Virchow's case showed both carcinomatous and sarcomatous nodules and had metastasized in the liver.

Contents of cysts in almost every case were a greasy semisolid material in which hair was mingled.

Diagnosis. — A positive diagnosis can be made only when portions of the cyst contents are obtained for examination.

This is possible when there is a fistulous communication between the cyst and the surface of the body, when through a communication between the cyst and a bronchus some of the contents is coughed up, or when an exploratory incision or trochar puncture is made. A probable diagnosis can be made when we have evidence, in a young adult, of a solid non-pulsating mediastinal tumor of slow progress. At the same time, owing to the rarity of this affection, it is not likely that a correct diagnosis will be made in the deeply seated tumors except where there is a history of having coughed up hair. This latter is absolutely pathognomonic of the condition, but in some of the previous cases this has been regarded as a deception on the patient's part and given no weight in determining the diagnosis.

Treatment. — It is only through surgical procedure that a cure can be attained. Radical operation is rendered difficult by the proximity of vital organs, the frequency of extensive adhesions, and the danger of entering the pleural cavity with the production of a pneumothorax. Simple drainage often proves ineffectual, owing to the cyst being multilocular or to the presence of diverticula in a simple cyst.

Of the cases collected eight were operated on. In six the cyst was incised and drained, with or without the removal of excrescences. Two of these cases resulted fatally. That of Koerte had concomitant pulmonary tuberculosis and died from hemoptysis. Mandlebaum's case survived for three weeks, though she never fully rallied from the operation. The remaining four were improved. Kückman's case, a year and a half after operation, showed only a small movable tumor, and the general condition was good. Pöhn's case was improved. Godlee's case after two years still showed fairly marked suffering. Pflanz's case was greatly improved, and when last seen, six months after the last operation, the fistula gave signs of permanently closing.

Two of the eight cases operated on were cured. In the case of Bastianelli a small cyst was excised with uninterrupted recovery. Dardignac's case was drained once by himself and a second time, four years later, by Belin. Each time

there was much improvement in the man's condition. Finally, one year later, Belin performed a partial pneumectomie and partial excision of the cyst with marsupialization of the remaining part. This resulted in complete recovery.

Though the number of cases is small, the results of operation have been fairly satisfactory. In those in which pressure symptoms are becoming very evident it seems that surgical interference should be undertaken, since without it a fatal termination is almost certain.

Genesis. — Few subjects in pathology have aroused more interest and discussion than the question of the genesis of those tumors, which are grouped under the designations, dermoid cyst and teratoma. To the earlier medical writers they represented, at least in certain parts of the body, the results of abnormal pregnancies inflicted by Heaven as punishment for some misdeed. In the latter part of the eighteenth century their origin was ascribed to a "nisus formativus," and this explanation was for a time deemed satisfactory. To Lebert is due the credit of separating the dermoid cyst, a name given by Leblanc,[48] from the teratoma. Wilms[49] has recently concluded that the dermoids of the ovary are structurally and genetically different from those occurring elsewhere in the body. The earlier theories, incompatible with our present knowledge of the pathology and embryology of man, have given place to newer ones. The later ones have this in common: they refer the origin of the tumor to the fetal period of life.

We will first consider the dermoid cysts, as they form a very large proportion of this class of tumors in the anterior mediastinum. Ribbert[50] has shown that portions of epidermis transplanted into the abdominal cavity can give rise to cysts lined by epidermis, and also[51] that bits of epidermis transplanted into the subcutaneous tissue will proliferate and line the split in the tissue in which they lie. If this is true for adult tissues whose power of reproducing themselves is diminished, misplaced fetal tissues should show far greater capability of development. That dermoid cysts do arise in this way is generally conceded. Are there then

any conditions of fetal development which would favor ektodermal misplacement in this region of the body?

As we have already pointed out, many of the cysts in the cases studied occupy that portion of the anterior mediastinum situated between the upper border of the heart and the root of the neck. Others give proof of a prior position here. We know that the heart develops in the neck region of the fetus, and later descends into the thorax. Such a descent would be expected for other structures in the superior anterior mediastinum. This does happen in the case of the thymus. It seems justifiable to assume a similar migration for the anlage of the dermoid cysts.

In the neck region during fetal life we have a cycle of changes involving the branchial clefts and arches, the formation and disappearance of the sinus precervicalis of His, the development of the thymus, and the thyroid from the third and forth branchial clefts respectively, and the overlapping of the fourth arch by the third. The sinus precervicalis is ektodermal, the thymus and thyroid are regarded as entodermal in origin by almost all observers. However, regarding the branchial clefts, Minot [52] points out the very intimate fusion between the ekto and ento dermal layers. Thus in the neck we have great complexity of development and conditions apparently favoring the misplacement of portions of germinal layers. In other parts of the body where dermoid cysts and teratomata are frequent we find similar complexity of development.

In the case reported by Marchand distinct thymus tissue was present. In one of Pinders' cases there was tissue suggesting thymus, and associated with the other was lymphosarcoma, whose origin in this region is generally referred to the thymus. In these three we have evidence of association in development with the thymus. That thymus tissue is not found in more cases may be due to the fact that the thymus normally disappears in early adult life.

Collenberg's case showed definite association with the thyroid, and in Mandlebaum's case vesicles were present identical with those normally present in the thyroid. Thus

in a certain number of instances we have proof of the genetic association of these tumors with thyroid or thymus.

Besides these cysts to which a branchiogenic origin may be assigned, there are cysts in the lower anterior mediastinum which have no demonstrable connection with the upper anterior mediastinum. They have either migrated from the upper level, and have the same origin as the cysts there, or they have resulted from portions of misplaced ektoderm caught within the thorax at the time of closure of the anterior chest wall. Moreover, this origin cannot be denied for some of the cysts at a higher level. Of it we have no definite proof, but the occurrence of presternal dermoid cysts of which Andoly [53] has collected seven cases somewhat favors this view, while the case of Bergmann, in which the sternum lies between two parts of the tumor connected by a fistula through the bone, strongly supports it.

For those cases in which were found cysts lined by ciliated epithelium, it is not necessary to assume any participation of the respiratory tract in their formation. Ciliated cysts can arise in the thymus, as in the case reported by Westernyk [54] and a second of Bednar's cited by the former. It is also stated that such cysts are not uncommon in animals.

The two cases, Ekehorn (Case I.) and Virchow, which have been regarded as teratomata show parts derived from all three germ layers. This class is at present generally regarded as originating from (1) a fetal inclusion, (2) from a fertilized polar body, or (3) from a group of cells separated from the developing ovum very early in segmentation. Though there is some evidence for these theories in experimental work on the lower forms of life, they remain scarcely more than theories for man. At the same time it is possible that the more complex tumors arise from misplaced elements representing all three germ layers, and this view would explain all cases as yet reported for the anterior mediastinum, since here the degree of complexity is not very great.

In conclusion it would seem that most of the tumors of the anterior mediastinum have a branchiogenic origin. For a certain number an origin resulting from the misplacement

of ektoderm during the closure of the anterior chest wall cannot be denied.

Summary.

1. Dermoid cysts and teratomata of the anterior mediastinum occur infrequently, generally give evidence of their presence during early adult life, and are of relatively long duration.

2. Their most frequent position is immediately behind the upper portion of the sternum. From there they may grow out into the lung or down between heart and lung.

3. These tumors may be classified as follows:

(*a.*) Tumors of ektodermal origin with the addition of some tissue from the mesoderm — dermoid cysts.

(*b.*) Tumors derived from all three germ layers — teratomata.

(*c.*) Dermoid cysts or teratomata which in some part of their structure show evident malignancy.

The first class includes a large proportion of the reported cases.

4. The coughing up of hair is pathognomonic of this mediastinal condition.

5. Cure is possible only through surgical procedure.

6. The genesis of these tumors is to be referred to the fetal period of life. The origin of most is branchiogenic. Some may result from germ layers misplaced at the time of closure of the anterior chest wall.

LITERATURE.

1. Hare, H. A. Mediastinal Disease. Phila., 1889.
2. Naumann. Schmidt's Jahrb., 1859, CIV., 301.
3. Lebert. Ueber Dermoidcysten. Prager Vierteljasch. f. d. prakt. Heilk., 1858, LX., 47. Ref. Schmidt's Jahrb., 1859, CIV., 301.
4. Finkler & Ribbert. Berlin Klin. Woch., 1887, 250.
5. Pinders. Ueber Dermoidcyste des vorderen Mediast. Inaug.-Diss., Bonn, 1887. (Cited by Pflanz & Ekehorn.)
6. Hoffmann. Erkrankungen d. Mediastinums. Nothnagel's Spec. Path. u. Therapie, 1896, XIII.
7. Pflanz. Ueber Dermoidcysten d. Mediastinum anticum. Zeitsch. f. Heilkunde, 1896, XVII., 473.

8. Spath. Tumeur du Mediastin anterieur composée de Tissu stéatomateux et mélicérique. Gaz. Med. de Paris, 1836, 120.
9. Ekehorn. Die Dermoidcysten des Mediastinum anticum. Arch. f. klin. Chir., 1898, LVI., 107.
10. Gordon. Case of Tumor in the Anterior Mediastinum containing Bone and Teeth. Med.-Chir. Trans., 1827, XIII., 12.
11. Mohr. Berlin Med. Centralzeitung, 1839, 261. (Cited by Ekehorn.)
12. Münz. Alber's Atlas d. Path. Anat., 1839.
13. Buchner. Deutsche Klinik, 1853, XXVIII., 311. (Cited by Cordes & Dardignac.)
14. Cordes. Dermocystoid im Mediastinum anticum. Virch. Arch., 1859, XVI., 290.
15. Cloetta. Ueber das Vorkommen einer Dermoidcyste in der Lunge. Virch. Arch., 1861, XX., 42.
16. Salomonsen. Eine Dermoidcyste in der Lunge. Ref. Schmidt's Jahrb., 1864, CXXIV., 292.
17. Collenberg. Zur Entwicklung der Dermoidkystome. Inaug.-Diss., Breslau, 1869.
Ein Fall von Dermoidkystom in vord. Mediastinalraume. Ref. Schmidt's Jahrb., 1870, CXLVI., 37.
18. Waldeyer. Dermoidkystom des vorderen Mediastinalraum. Arch. f. klin. Chir., 1871, XII., 843.
19. Nobiling. Bayr. Artzlich. Intelligenzblatt, 1869. (Cited by Ekehorn.)
20. Villard. (Case cited by Dardignac.)
21. Pöhn. Beschreibung eines Falles von Dermoidcyste des Mediastinum anticum. Inaug.-Diss., Berlin, 1871. (Cited by Pflanz & Ekehorn.)
22. Virchow. Teratoma myomatodes mediastini. Virch. Arch., 1871, LIII., 444.
23. Kückmann. Anlegung einer kunstlichen Cystenfistel zur Heilung einer Haarbalgsgeschwulst in Mediastinum anticum. Inaug.-Diss., Marburg, 1874. (Cited by Pflanz & Ekehorn.)
24. Gläser. (Cited by Danzel.)
25. Danzel. Geschwulst mit Haaren im Rectum. Arch. f. klin. Chir., XVII., 443.
26. Marchand. Beiträge zur Kenntniss der Dermoid geschwülste. 22d Bericht der oberhessichen Gesellsch. f. Natur u. Heilkunde, Giessen, 1883, 325. (Cited by Pflanz & Ekehorn.)
27. Paget. Surgery of Holmes & Hulke, 1883. (Cited by Dardignac.)
28. Loewenmeyer. Dermoidcyste des Mediastinums. Berlin. Klin. Woch., 1888, 135.
29. Sangalli. Grossa ciste colloidea composta del polmone destro con peli, glandole sebacee e lamine ossee nelle sue pareti. Rendiconti del R. Instituto Lombardo, 1888, XXI., 662. (Cited by Ekehorn & Pflanz.)
30. Godlee. Dermoid Cyst of the Right Side of the Chest communicating with a Bronchus. Med.-Chir. Trans., 1889, LXXII., 317.
31. Sormani. Gazetta degli Ospitale, No. 40, 1890. (Cited by Pflanz.)
32. Marfan. Kyste dermoide der Mediastin anterieur. Gaz. Hebd. de Med. et de Chir., 1891, 394.

33. Koerte. Berlin. Klin. Woch., 1891, 1204.
34. Harres. Ueber Zahne in Dermoidcysten. Inaug.-Diss., Zurich, 1892. (Cited by Ekehorn.)
35. Jores. Uber die Verbindung einer Dermoidcyste mit malignen Cystosarcom. Virch. Arch., 1893, CXXXIII., 66.
36. Bastianelli. Riforma medica, May 20, 1893. (Cited by Pflanz)
37. Dardignac. Tumeur dermoide du Mediastin anterieur. Rev. de Chir., 1894, XIV., 776.
38. Belin. Observation d'un cas rare de Kyste dermoide du Mediastin. Pneumectomie partielle. Guérison. Clermont (Oise), Imp. Daix fres., 1900, 8°, 23.
39. Smythe. Dermoid Cyst of Lung. Amer. Pract., 1879, XIX., 313.
40. Jones. British Med. Jour., 1880, I., 286.
41. White. Dermoid Cyst attached to the Front of Pericardium. Trans. of Path. Soc. Lond., 1890, XLI., 283.
42. Kretz. Beitrag zur Casuistik der Dermoide. Wien. Klin. Woch., 1893, VI., 861.
43. Ogle. Trans. of Path. Soc. Lond., 1897, XLVIII., 37.
44. Bergmann. Ueber ein Fall v. Dermoidcysten der vorderen Mediastinum. Prag. Med. Woch., 1898, XXIII., 109.
45. Mandlebaum. A Case of Dermoid Cyst of Anterior Mediastinum. Am. Jr. of Med. Sc., 1900, XX., 64.
46. Nederland Weekblatt von Geneesk, 1857, XLIV. (Cited by Hare.)
47. Harris. St. Barth. Hosp. Reports, XXVIII., 73.
48. Leblanc. Sur une Kyste dermoide du Crâne. Jour. de Med. veterinaire, II. (Cited by Wilms.)
49. Wilms. Ueber d. Dermoidcysten und Teratome, mit besonderer Berücksichtigung d. Dermoide d. Ovarien. Deutsch. Arch. f. klin. Med., 1895, LV., 289.
50. Ribbert. Allgemeine Path. u. allg. path. Anat., Leipzig, 1901, 524.
51. Ribbert. Loc. cit., 285.
52. Minot. Human Embryology. New York, 1892, 265.
53. Andoly. Contribution à l'Étude des Kysten dermoides de la Region presternal. Paris, 1898, 8°, No. 635.
54. Westernyk. Zur Casuistik d. med. Cysten. Prag. Med. Woch., 1900, XXV., 373.

THE MOVEMENTS OF THE INTESTINES STUDIED BY MEANS OF THE RÖNTGEN RAYS.

W. B. CANNON.

(From the Laboratory of Physiology in the Harvard Medical School.)

THE METHOD. — The method used in this research is the same as that used in investigating the movements of the stomach[1]. Bismuth subnitrate, ten to thirty-three per cent., mixed with the food, causes a shadow of the intestinal contents to be cast on the fluorescent screen. Thus movements of the intestinal contents and thereby movements of the intestinal wall are rendered visible. These observations have been made on the cat.

MOVEMENTS OF THE SMALL INTESTINES. — The activity most commonly seen in the small intestine is the simultaneous division of the food lying in a loop into small segments, and a rhythmic repetition of the division without any notable advance of the food through the gut. I have called this process the rhythmic segmentation of the intestinal contents. It consists essentially in the appearance of constrictions at regular intervals along the length of a mass of food, which cut the mass into little ovoid pieces, — a series of uniform segments. (See Fig. 1, line 2.) A moment later each of these segments is divided into two particles, and immediately after the division neighboring particles (as a and b, line 2, Fig. 1) rush together and merge to form new segments (as c, line 3, Fig. 1). The next moment these new segments are divided, and neighboring particles unite to make a third series, and so on.

In the cat this rhythmic segmentation may proceed at the rate of thirty divisions per minute, and as the process usually lasts for half an hour or more, it is clear that the food may be divided in the manner above described nearly a thousand times without the appearance of peristalsis.

[1] Cannon: Journal of Boston Society of Medical Sciences, February, 1898.

The constrictions causing the segmentation thoroughly mix the food and digestive juices, and bring the digested food into contact with the absorbing mechanisms. Moreover, since Mall has shown that contraction of the intestinal wall serves to empty the venous and lymphatic radicles, it is clear that these rhythmic constrictions also act in deporting through blood and lymph channels the absorbed material.

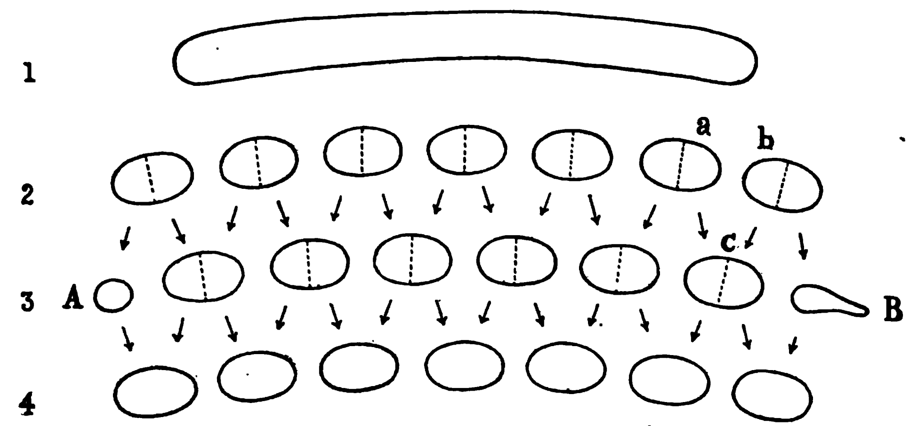


FIGURE 1.—A diagram representing the process of rhythmic segmentation of the intestinal contents.

Peristalsis is usually combined with segmentation. As the food is advancing, interfering constrictions often separate the rear end of the mass from the main body. The separation is momentary, however, and the rear end is swept into union with the main body again, and the whole mass is pushed onward until another constriction repeats the changes.

THE COMPETENCE OF THE ILEOCECAL VALVE.—The ileocecal valve is thoroughly competent for the food entering the colon from the ileum. This is proved not only by the impossibility of forcing food back from the large into the small intestine by pressure through the abdominal wall, but also by a similar ineffectiveness of the activity of the ascending and transverse colon and the cecum.

THE MOVEMENTS OF THE LARGE INTESTINE. — The usual movement of the transverse and ascending colon and the cecum is antiperistalsis. This activity recurs in periods about fifteen minutes apart, and each period lasts commonly about five minutes; the waves run during a period at the rate usually of eleven waves in two minutes. This antiperistalsis gives new significance to the ileocecal valve; for the food, now in a closed sac, is thoroughly churned and mixed by the constrictions running towards the cecum and again exposed to absorbing walls, without any interference with the processes in the small intestine.

As soon as new food enters the large intestine, a strong general contraction takes place along the cecum and ascending colon, forcing some of the food onward. A moment later, however, antiperistaltic waves begin to pass over the region and fill it again to normal fulness.

As the food is forced onward it accumulates in the transverse colon. With the accumulation deep tonic constrictions appear one after another and carry the material slowly into the descending colon, leaving the transverse and ascending portions and the cecum free for the antiperistaltic waves.

DEFECATION. — In emptying the large intestine the material in the lower descending colon is first carried out by combined peristalsis and pressure of the abdominal muscles; the remainder of the material is then spread into the evacuated region, and this region is again cleared; the second remainder may be similarly spread into the newly evacuated region. In normal life the new food arriving in the colon must force forward the old contents of the ascending and transverse colon, just as the later parts of a meal force forward the earlier parts.

THE QUESTION OF ANTIPERISTALSIS. — The observations have revealed no evidence of antiperistalsis in the small intestine, but since the ileocecal valve will allow nutrient material, if under pressure, to pass backward, the antiperistalsis of the ascending and transverse colon may force into the

small intestine a considerable portion of a nutrient enema filling the large intestine. Segmentation in the small intestine affects such an enema precisely as it affects food which has passed normally through the stomach.

THE EFFECT OF EMOTIONS AND SLEEP. — Signs of emotion, such as fear, distress, or rage, are accompanied by a total cessation of the movements of both the large and small intestines. The movements continue in the cat during sleep and at night.

LEUCOCYTOSIS AFTER VIOLENT EXERCISE.

RALPH C. LARRABEE.

The paper is based on a study of the blood of four of the contestants in the Boston Athletic Association's Marathon race of 1901. This is a road race of about twenty-five miles (40 kilometers), held each spring. The severity of the contest will be apparent when it is said that the winner — not included in my four — covered the distance in less than two and one-half hours. This is about ten miles an hour, about as fast as an ordinary man rides his bicycle for pleasure. In making the white counts and in collecting the blood I was assisted by Dr. W. H. McBain. The white counts were made with the Thoma-Zeiss apparatus. For the differentials one thousand white corpuscles were counted in each of the specimens collected after the race and five hundred in each of the normal ones collected before. Our results are shown in Table I.

The blood of these four cases before the race showed no abnormalities. The percentages of polymorphonuclear neutrophiles may perhaps run a little high, but this is to be expected in active young men in the best possible physical condition.

After the race the blood was taken immediately, within five minutes from the actual finish. In every case a leucocytosis was found, varying from 14,400 to 22,200. The differential count showed that the increase was mainly in the polymorphonuclear neutrophiles. The relation between the large and small mononuclear forms was changed, the proportion of large forms being increased. Eosinophiles were both relatively and absolutely diminished. In three of the four cases a few myelocytes were found. By myelocytes we mean mononuclear cells with neutrophilic granules, without reference to any particular theory as to their origin, and without intending to imply that they are or are not identical with the myelocytes of leukemia.

TABLE I. THE FIGURES IN PARENTHESES REFER TO THE ACTUAL NUMBERS OF CELLS PER CUBIC MILLIMETER.

NAME.	Before Race.							Immediately after Race.						Remarks.
	Interval.	Total Leucocytes.	Polymorph.	Small Mononuclear.	Large Mononuclear.	Eosinophile.	Myelocytes.	Total Leucocytes.	Polymorph.	Small Mononuclear.	Large Mononuclear.	Eosinophile.	Myelocytes.	
H—	3 days before	(9,800)	69% (6,762)	22.4% (2,195)	8.0% (784)	0.6% (59)	0	(14,400)	88.5% (12,744)	7% (1,008)	4.4% (634)	0	0.1% (14)	
	At start	(4,800)	72.8% (3,494)	18.2% (874)	8.2% (394)	0.8% (38)	0							
L—......	2 days before	(5,800)	63.2% (3,666)	26.8% (1,554)	8.2% (476)	1.8% (104)	0	(16,200)	90.3% (14,629)	4.5% (729)	4.4% (713)	0	0.8% (130)	Many cells intermediate between polymorphonuclears and myelocytes.
M—	At start	(3,700)	72% (2,664)	14.6% (540)	10.6% (392)	2.6% (96)	0.2% (7)	(20,800)	83.8% (17,430)	7.8% (1,622)	8.2% (1,706)	0.2% (42)	0	
P—......	3 days before	(8,200)	74% (6,068)	18.4% (1,509)	5.6% (459)	2.0% (164)	0	(22,200)	86.1% (19,114)	6.6% (1,465)	7.1% (1,576)	0	0.2% (44)	Few cells intermediate between polymorphonuclears and myelocytes.

The myelocytes were probably more numerous than the table would indicate, as we counted as polymorphonuclears a good many cells having neutrophilic granules, and single but more or less indented nuclei. In fact one specimen showed numerous cells that appeared to be intermediate between polymorphonuclears and myelocytes, concerning whose classification there was considerable doubt. No abnormalities were noticed in the red corpuscles.

In commenting upon these results we must bear in mind that leucocytosis may be of two sorts:

(1.) An increase in all the forms of white corpuscles so that their relative numbers are unchanged — the "physiological leucocytosis" such as is seen during digestion or after parturition.

(2.) Leucocytosis involving only or mainly the polymorphonuclears, the so-called "inflammatory" type, seen in many infections and toxic conditions.

According to Cabot[1] violent exercise gives a leucocytosis of the first type, usually explained by concentration of the blood from vaso-motor contraction and increase of blood-pressure. Shultz[2] holds that the increase is due to greater rapidity of circulation carrying corpuscles into the general arterial system that had been at rest in the greater abdominal veins. According to the same author, the number varies up to 13,600, which was his highest figure. Obviously our cases do not come under this heading. In each of the four runners the polymorphonuclear cells are increased out of proportion to the other forms, and again the degree is far above Shultz's. Moreover in our cases after the race the blood-pressure was decreased. The exertion had gone far beyond physiological limits and our results certainly show that where this is the case we may get a considerable leucocytosis of the inflammatory type.

A close correspondence exists between our results and those obtained by F. G. Burrows[3] in a study of the leucocytosis associated with convulsions. As in our cases, he found a leucocytosis conforming both in degree and in preponderance of the polymorphonuclear forms to the inflammatory

type. As in our cases he also found relative and absolute decrease of the eosinophiles and the appearance of a few myelocytes. He found reasons for supposing that the leucocytosis was the result of a double cause — first a moderate increase due purely to the muscular work of the convulsions and added to this a leucocytosis of an inflammatory or toxic nature. Where both causes acted together a higher leucocytosis would result than from the toxic cause alone, but the percentage of polymorphonuclears would be less than where the increase was purely inflammatory. From the study of a case of general paralysis with violent frenzy, but no convulsions, and of a healthy young athlete after a short violent run, he infers that muscular work alone is not capable of producing leucocytosis of the inflammatory type. Our figures, however, certainly prove that this inference was not justifiable; muscular work alone, if sufficiently violent and prolonged, can produce leucocytosis of the inflammatory type.

The question then arises, May not the increase of white corpuscles in our cases be due to a double cause: muscular work acting mechanically and producing a physiological leucocytosis plus a toxemia acting chemically to produce an inflammatory leucocytosis? Looking at our figures once more it will be noticed that the two highest white counts are in the cases showing the lowest percentage of polymorphonuclears. This is what we should expect if the lower counts were due to increase of the polymorphonuclears alone or mainly, while the higher were made up of the same thing plus a further increase involving all the forms about alike.

Since the toxic portion of the leucocytosis involves only the polymorphonuclear neutrophiles, let us assume that the increase in total mononuclears (large and small) indicates the degree of increase of all forms alike. In the case of H—— the total of mononuclears just before the race was twelve hundred and sixty-eight, and after the race sixteen hundred and forty-two. It is a simple arithmetical problem to find that if all forms of leucocytes were increased in this ratio the leucocytes at the finish would number sixty-two hundred and fif-

teen, an increase ("physiological" in type) of fourteen hundred and fifteen (= 6,215 — 4,800). The actual count was fourteen thousand four hundred, so that eighty-one hundred and eighty-five (= 14,400 — 6,215) more polymorphonuclears must have been added, over and above the general increase. In other words, the fourteen thousand four hundred leucocytes found at the finish consisted of the original forty-eight hundred, an additional fourteen hundred and fifteen of physiological type, and eighty-one hundred and eighty-five more from toxic causes. I use the count made at the start. By using the same method on all four cases we made up the accompanying chart (Table II). In L——'s case there is an absolute *loss* of mononuclears, assumed to occur "all along the line."

TABLE II.

	H——.	L——.	M——.	P——.
Loss of weight during race	5¼lbs.	4¼lbs.	4lbs.	2½lbs.
Physiological leucocytosis..............	+1,415	—1,680	+9,512	+4,470
Toxic leucocytosis	+8,185	+12,080	+7,588	+9,530
Original number of leucocytes	4,800	5,800	3,700	8,200
Total leucocytes......	14,400	16,200	20,800	22,200

The value of the table is greatly decreased by several facts. In two cases we had to use counts made several days before the race. Again the change in relative numbers of large and small mononuclears throws doubt on the propriety of using the total mononuclears as indicators of a like increase in all forms of leucocytes. Finally it is perhaps more than questionable whether we have a right to assume sufficient mathematical exactness in these blood changes to give such a table even approximate accuracy.

It will be noticed that the physiological element varies greatly, but that the toxic part is fairly constant, strikingly so if we omit the case of L——, which shows a puzzling decrease of mononuclears.

These results become clear and harmonize with those of Burrows and of Shultz, if we assume that there are three stages in the blood changes due to severe, prolonged, exhausting work. (1) A stage where there is a simple physiological leucocytosis — an increase "all along the line." That this exists Shultz and others have demonstrated. (2) A stage where, in addition to this, there is also an increase of the inflammatory type, probably due to toxic causes. That such a double cause may exist Burrows has shown. The count is here highest of all. (3) A final stage where, owing to extreme exhaustion, the physiological increase has disappeared, leaving only the toxic. Here the total is less high than in the second stage, but the proportion of polymorphonuclears is higher. That the physiological leucocytosis in convulsions is temporary, and may subside while its cause continues, Burrows has shown. It is probable that in our cases also the physiological leucocytosis is temporary.

Of our four cases all had passed the first stage. M—— and P——, with high total white corpuscles and relatively low percentages of polymorphonuclears, were in the second stage, while H—— and especially L——, with low totals and relatively higher polymorphonuclears, were in the third. Incidentally it may be said that of the four men M—— was in the best, and L—— in the poorest, condition at the finish.

We are aware that the results here stated depend upon the manipulation of figures, perhaps to an unjustifiable extent, for the changes do not occur with mathematical precision. Too few cases were studied to justify final conclusions. Excitement and exposure to cold probably have a hand in the results. It is probable that the count is affected by concentration of the blood from sweating, though all drank water during the run. All the men lost weight, but the physiological increase of leucocytes was not, as the table shows, in proportion to this. The subject demands further study.

The disappearance of the eosinophiles has been observed by others in leucocytosis from various diseases. As Dr. Cabot has pointed out to the writer, its importance here is that it makes the comparison with the leucocytosis of diseased conditions closer. Mechanical changes pure and simple might increase the absolute numbers of the different forms of leucocytes differently, but would hardly account for an absolute *decrease* in one form alone. The change in proportionate numbers of the small and large mononuclears has also been previously noted.

The occurrence of myelocytes has been noticed both by Burrows[3] and by Capps[4] in convulsions. The latter found, in a general paralytic, who had had an apoplectiform attack and was dying with extreme cyanosis and dyspnea, twelve and six-tenths per cent. of mononuclear neutrophiles, besides a number of cells showing every gradation between such and the ordinary polymorphonuclears. His description of this blood would apply to one of our cases. Such observations are of importance in connection with the theory that myelocytes give rise to polymorphonuclears by changes in the shape of the nucleus.

CONCLUSIONS.—Violent, prolonged, exhausting work produces a leucocytosis.

This leucocytosis is made up principally by an increase in the polymorphonuclear cells, but the other forms may also be considerably increased in numbers.

More than one cause acts to produce the leucocytosis—probably a temporary, mechanical cause, and a toxic cause, more slow to develop, but lasting as long as the exercise continues.

REFERENCES.

1. Cabot. Clinical Examination of the Blood, 1897. Pages 87 and 92.
2. Shultz. Deut. Arch. f. klin. Med., 1893. Page 234.
3. Burrows. Amer. Journ. Medical Sciences. May, 1899.
4. Capps. Amer. Journ. Med. Sciences. June, 1896.

TUBULAR PERIVASCULAR SARCOMA. ITS ORIGIN, STRUCTURE, AND METASTASIS.

H. C. Low, M.D.
(*Late First Assistant in Pathology in the Boston City Hospital*),

AND

F. B. Lund, M.D.

The study of the specimens removed at operation and autopsy in the two cases of perivascular sarcoma which are here reported, brought out so many points of interest in regard to the structure of the tumors, the changes in structure which may accompany metastasis, and the connection between the pathological findings and the clinical course of the disease, that the writers thought them worthy of the following report:

METHODS. — Of the original growth in the first case we have had to make our study from the sections made at the time and stained in hematoxylin and eosin. Of the recurrence at the primary site and the tumor removed from the groin at the third operation, all the tissue was saved in Zenker's fluid. At the autopsy all of the tumor and its metastases were preserved in Zenker's fluid, except small nodules in the brain which were saved partly in Zenker's fluid, but chiefly in alcohol and formalin.

From these tissues subsequently imbedded in paraffin over five hundred sections have been cut from various parts of the tumor and its metastases. The stains used have been Mayer's hemalum with eosin as a contrast, Unna's polychrome methylene blue, Mallory's connective tissue stain, and Weigert's elastic tissue stain.

The tissue and sections of the first two operations in the second case were not saved, and we use the very full description taken from the pathological records. The tissue removed at the third operation, consisting of the recurrence at the original site on the penis and the metastasis in the groin,

was preserved in Zenker's fluid, and from this we have made numerous sections.

CASE I.—E. R., forty-seven years of age, married, multipara, who had just passed the climacteric, was admitted to the service of Dr. Munro at the City Hospital on September 13, 1899. About four or five months before, she had noticed a smarting sensation on micturition as the urine passed a small bunch close to the outlet of the urethra. The growth had not apparently increased in size since she noticed it, but it was tender and painful on sexual intercourse.

Examination showed a small bright-red eroded tumor, close to the urethra, and between it and the right labium minus. There were also pigmented areas the size of a ten-cent piece on the inner surface of both the right and left labia minora. Except for pigmentation these areas of skin appeared normal. On September fifteenth, under local anesthesia with cocaine, one of the writers excised the tumor and closed the wound with silk sutures.

The pathological report at the time of the first operation, September 19, 1899, describes the gross appearances as a simple bit of tissue with a small dark elevation in the side which is covered with skin. The diagnosis was made as follows:

> Caruncle with chronic inflammation. At one point is a small area resembling the pigmented warts frequently found beneath the skin, and which, although they histologically resemble sarcomata, are clinically but slightly or not at all malignant.

Owing to the fact that the clinical appearance of the growth was not that of a malignant tumor, but that of an ordinary urethral caruncle, and that the pathological report confirmed the surgeon in the belief of the non-malignancy of the tumor, no further operation was undertaken. That this course was a mistaken one the subsequent course of events clearly proved.

The microscopical section (Plate III., Figure 1) shows rather dense and fairly vascular fibrous tissue bordered along one side by epidermis. In the middle of this side is a nearly round nodule partly covered with a thin

epidermis. About this nodule, and especially just beneath the adjacent epidermis, there is a marked infiltration of lymphoid and plasma cells. There is at one side a slight ulceration with a few polymorphonuclear neutrophiles. The nodule itself, which in this section is about two millimeters in diameter, is formed of round and spindle-shaped cells with nearly round or oval nuclei. The cytoplasm is finely granular and takes a light stain; the nuclei are lightly stained, contain many chromatin granules, and have a clearly defined membrane. There are many very typical mitotic figures in various stages.

There is a small amount of stroma between the cells, and in places it is formed into narrow bands which irregularly divide the small cell groups into more or less definite alveoli. At one side near what appears to be a growing edge there is a well-marked alveolar arrangement.

There is in places considerable granular pigment. This is not in any one part of the tumor, but is gathered in masses sometimes in the tumor cells, but more often between these cells and in the connective tissue. Along the borders of the tumor this pigment is mostly scattered about in the tumor cells. Occasionally, in the center of an alveolar group of cells, is seen a large cell with irregular outline in which the cytoplasm is dotted with numerous fine round brown granules. Some of the cells, which at least in places seem to be those of the stroma, are filled with larger irregular and darker brown granules. Where the pigment is between the cells the granules are larger and darker. The nuclei do not seem to be pigmented. The pigment cells are mostly of a long irregular spindle-shape, and when nearly round often show delicate projections extending between the adjacent cells. The nodule is not very vascular, and no definite vessels are seen.

On August 30, 1900, she was admitted to the hospital again in Dr. Munro's service. The upper halves of both labia minora, the vestibule, and the clitoris were occupied by a bluish-black, elevated, irregular new growth which at the slightest touch bled freely from an eroded surface near its center.

On August thirtieth, under ether, an incision was made by one of the writers, extending from the top of the right labium majus outside the tumor downward to below the lowest black spot, then upwards along the inner surface of the labium minus to just above the urethra, then across close to the roof of the urethra, which was defined by a catheter, then around the involved portion of the left labium minus. The very vascular tumor with the involved portions of the labia minora and the clitoris were removed, care being taken to cut wide

of the growth. After tying numerous vessels the wound was sutured in the shape of an inverted Y. No enlargement of the inguinal glands was detected. Healing was by first intention.

The pathological examination of the specimen removed at the second operation, eleven months later, August 31, 1900, is as follows: The specimen is a piece of tissue, six and one-half by four by one centimeters, consisting of skin from the labia and subcutaneous tissue. It contains on the surface two nodules, one elevated one centimeter with a base two by three centimeters; the other elevated about one-fourth of a centimeter, with a base one-half of a centimeter in diameter. Both are very darkly pigmented. There are in addition several areas of diffuse subcutaneous pigmentation, not indurated, and apparently non-inflammatory, and not elevated above the surface. The diagnosis then made was alveolar melanotic sarcoma.

Section through the *larger* nodule shows a growth surrounded by very dense fibrous tissue and divided by well-marked bands of connective tissue into a number of large lobules; these are further divided by bands of connective tissue into smaller irregular areas. In most of these areas is a definite alveolar arrangement (Plate III., Figure 2), but there is everywhere a small amount of stroma between the individual cells. The alveoli vary widely in size and shape, some being long and narrow, others large and irregular. In many places the alveolar arrangement becomes very indefinite, only to assume a more typical character near by. Occasionally long bands of spindle-shaped cells run through the section, breaking up this alveolar arrangement.

The cells in the alveolar part are oval or moderately elongated, except for an occasional band of typical spindle-shaped cells. These are in general fairly large, and rarely a large one is seen with two or three nuclei. The cytoplasm is lightly stained and very finely granular. The nuclei are large, irregular, and slightly elongated; they are vesicular, have a definite nuclear membrane and numerous fine chromatin granules. Many show a very definite nucleolus with fine radiating chromatin threads. Mitotic figures are numerous and beautifully preserved. There is no pigment in the alveolar portion. In this part of the tumor the blood vessels lie in the broad bands of stroma and have no intimate relation to the tumor cells.

In a part of the section the alveoli become irregularly elongated and separated by indefinite fan-shaped bands of connective tissue and then disappear altogether. In a large part of the tumor there is no alveolar arrangement, except that running through the tumor there are occasional

bundles of narrow fibrous tissue bands enclosing long groups of cells. The whole nodule is surrounded by dense fibrous tissue. The cells in this part of the tumor differ only slightly from the others in the fact that they tend more towards a spindle-shape. Mitosis is less noticeable. Many of these cells are pigmented, chiefly those in and around the fan-shaped bands of connective tissue. Most of the pigment is, however, distributed in larger granules between the cells. This part of the tumor is not very vascular and the few vessels noticeable in it do not seem to form an integral part.

This large nodule, as the gross description shows, spreads over the surface for two to three centimeters. The epithelium has disappeared and the surface of the tumor shows some evidence of a chronic inflammatory process. In the adjacent tissue there is marked increase of connective tissue and infiltration of plasma cells and polymorphonuclear leucocytes. Although at its base the tumor is limited by a dense wall of fibrous tissue, its lateral borders are ill-defined. Along the edges the tissue is very vascular and resembles granulation tissue.

The smaller nodule, about half a centimeter in diameter, lies just beneath the very thin layer of epidermis which remains. There is considerable stroma throughout the tissue and no tendency to alveolar arrangement. The tumor is not at all vascular. The cells are mostly elongated or typical spindle-shape; some are moderate-sized round cells. They have nearly the same character as the cells of the rest of the growth. Their nuclei, however, are more lightly stained and do not commonly show a nucleolus or numerous chromatin granules. A few cells show typical mitotic figures.

The only pigment in the main part of this nodule is in a few scattered tumor cells. At one side, where there is extensive invasion of the adjacent connective tissue, there is an extraordinary amount of pigment. This is beneath a slightly hypertrophied epithelium, and the cross-sections of some of the papillæ of the rete Malpighii are filled with pigment-bearing cells. From just beneath the epithelial layer these pigment cells fill the subjacent tissue down to the base of the tumor nodule. The pigment cells are mostly very much elongated cells, which lie together, forming bands of stroma with the large tumor cells between them. In places there are many of the larger round tumor cells filled with pigment. The pigment is in rather large dark brown granules, which vary much in size and shape. There is little pigment between the cells. There is nowhere any hemorrhage into the tissue and no particular vascularity of the tumor or adjacent tissue, so the pigmentation does not seem to be from that source.

The patient returned to the hospital on February 19, 1901, stating that three or four weeks after her discharge from the hospital, which was on September 17, 1900, she noticed a small swelling in the right groin, which gradually increased

in size, and two months ago she had noticed a tumor in the left groin, which was also growing.

On examination a mass was found in the right groin lying beneath Poupart's ligament and projecting above and below it, which was fixed apparently to that ligament and to the aponeurosis, but not adherent to the skin. Owing to the shape of the tumor it was thought that it could best be removed by a curved incision with the convexity upward, the idea being that the blood supply would come from below through the superficial epigastric artery. On dissecting down to the aponeurosis, a very large mass was found, consisting apparently of all the inguinal and femoral glands, some of which were fused into a soft vascular, pigmented mass, the thin capsule of which burst during removal, allowing the escape of a soft gelatinous material. The deepest part of the mass occupied the saphenous opening and was firmly adherent to, if it did not involve, the anterior wall of the saphenous vein at its entrance into the femoral. An opening was made in the anterior wall of the vein, which bled freely, but was caught in two hemostats and puckered up by a catgut ligature. After tying the blood vessels, with which the tumor was freely supplied, the aponeurosis of the external oblique muscle and the fascia lata, which were widely exposed, was inspected and found to be clean, and apparently free from diseased tissue. Through an oblique incision the left inguinal glands, some of which were softened, melanotic, and enlarged to the size of an English walnut, were dissected out, and also two pigmented patches removed from the inner surface of the labia majora.

The patient left the hospital in about six weeks, the wounds having healed except for a sinus, and returned to the Out-Patient Department for treatment, where she continued until the sinus was healed.

The description of the gross appearances of the tissue removed at the third operation (February 23, 1901), seventeen months after the first operation, is as follows: A piece of skin two by one and one-half centimeters, which presents a very slightly elevated blue-black ramifying figure; the

pigmented area is sharply circumscribed in outline and of the same consistency as the rest of the skin. From the left groin, a mass of fat in which are imbedded three rather firm nodules, closely bound together. On section the nodules are deep bluish-black in color and on pressure yield a thick inky fluid. One nodule, one centimeter in diameter, is rather lighter in color, having a reddish tinge. The largest nodule, two by two and eight-tenths centimeters, is softened at the center. Detached from the above-mentioned masses is a nodule one-half centimeter in diameter, similar to the larger ones. There is a black discoloration running away from the nodules through the fat tissues. The third specimen is from the groin on the right side and consists of a mass of fat tissue and clotted blood surrounding an oval tumor five by three and one-half by four centimeters which for the most part is fairly definitely encapsulated, but in one or two places extends out into the surrounding fat tissue. On section it appears greenish-red and very soft. The cut surface is granular. The tissue pulls out like the tubules of an edematous testicle. These threads are about one to two millimeters in diameter and often pull out one to two centimeters in length.

An examination of the tissue after hardening in Zenker's fluid shows several irregular cysts with a firm capsule, lining which there is more or less soft tumor tissue and a fringe of very numerous smooth threads which with the hemorrhagic intercolumnar material filled these cavities like a bag of thread worms.

The sections of the tissue from the left groin show three different types of growth in the lymph nodes. In the smallest, where the metastatic tumor is of youngest duration, we find a node of rich cellular lymphoid tissue. The follicles are large and consist chiefly of plasma and lymphoid cells. Some of their cells are phagocytic and occasionally very large and vesicular. Between these is a rich lymphoid tissue with a moderate amount of stroma. Running in from the side between the follicles are bands of elongated cells with long lightly stained nuclei. These are not typical spindle cells, but look like rich proliferating connective-tissue cells, and none of them are pigmented. In places they are scattered in the lymphoid tissue. Two rather doubtful mitotic figures were found. The tissue is not very vascular, and there is no sign of proliferation of the endothelium of the blood vessels. There are, however, irregular spaces with no lining endothelium, which contain blood corpuscles and lymphoid cells and occasional large cells with a large irregular lightly stained nucleus.

These cells have more or less brown pigment in fine granules. These cells, often with quite brown pigment, are also found scattered about singly in the lymphoid tissue. Just outside of the lymph nodes some were seen in the lymph vessels. Near one end of the lymph node many pigment cells occur singly and in small clumps. They have nearly round, lightly stained nuclei. and karyokinesis, if it exists, is hidden by the pigment. There is considerable rather edematous stroma in the part where these tumor cells are most numerous.

About half a centimeter distant is another lymph node in which there is but little lymphoid tissûe left. Nearly the whole of it is invaded by tumor cells with no definite arrangement, inclosing here and there small islands of lymphoid cells. There are very few blood vessels, and they seem to have no relation to the tumor cells. There are between the tumor cells many lymphoctyes and connective-tissue cells. The cells vary extremely in size and shape; a few of them are elongated; they are all deeply pigmented with rich brown pigment in large granules. The nuclei, which are mostly hidden by the pigment, are medium-sized, round, and lightly stained. Mitotic figures are not seen. There are numerous almost perfectly round empty spaces in the masses of tumor cells. The smallest of these seem to be formed by several of the pigmented cells each offering a flat or concave side to help in forming the well-defined border. Some of the larger and better-formed spaces seem to have a very delicate lining membrane and one or two of the connective-tissue cells of the stroma seem to have a part in their formation. None of them appear to have any wall of endothelial cells and they are all empty.

Near by is a larger lymph node completely invaded by the tumor. About the periphery the tissue consists mostly of large rounded tumor cells of the epithelioid type with large vesicular nuclei. Few of them show mitotic figures and rarely are they pigmented. There is some stroma to be seen between all the cells. but it is mostly arranged in narrow poorly defined bands, which to a certain extent divide the tumor into alveoli. Near the center this alveolar arrangement is well marked, and there is almost no intercellular tissue in the groups of tumor cells, and the bands of stroma are broader. Here it is noticeable that the tumor cells show very many more mitotic figures, and that none of them are pigmented. There is no evidence of a central vessel in these alveoli.

In the center of the node there is a rich connective tissue of elongated cells radiating in broad bands to the periphery. Enclosed in this stroma are deeply pigmented cells of all sizes and shapes, many quite long and some very large. Throughout this tissue there are still many lymphoid cells. The blood vessels, which are not very numerous, are surrounded by a slight edematous tissue, and the whole stroma is rather edematous. There are occasional spaces surrounded by pigmented cells like those in the nodule just described.

At one side these pigmented cells extend out into the more alveolar part of the growth, and are scattered among the non-pigmented cells of the growth. They seem to differ from these only in their large size and varied

form and the occasional multiplicity of their nuclei, and the fact that they do not seem to show mitotic figures. Often a large pigmented cell has become phagocytic and included a smaller cell and, rarely, one of the non-pigmented tumor cells is phagocytic.

In two or three instances one of these large pigmented cells surrounded an empty vacuole. These suggest the vaso-formative cells of the *sarcoma angioplastique* as described by Malassez and Carnot.

The medium-sized nodule removed shows a growth which at first glance appears to be of an entirely different type. Except at one point there is no lymphoid tissue. The tumor growth is definitely separated from this by a capsule of loose fibrous tissue, in the meshes of which there are great numbers of pigmented cells, forming a tissue resembling the center of the nodule just described. The new growth consists of masses of cells showing no definite arrangement. There are slits and irregular spaces everywhere, mostly empty, but rarely containing blood corpuscles. There is a little stroma between the individual cells. Running in every direction are narrow bands of connective tissue which divide the tumor into poorly defined lobules. In these bands there are often blood and lymph vessels. The cells seem to cluster more or less closely about the borders of these lobules, and in the center there is rarely a small blood vessel or an irregular space partly filled with blood. The cells are spindle-shaped and multiform; they are mostly large and have a single or, rarely, two or three very large, vesicular nuclei with a well-defined nucleolus. Many show mitotic figures. A great part of the cells are very lightly pigmented with fine granules. At the borders of the lobules and in the bands separating them are a great number of large cells deeply pigmented with coarse brown granules. Phagocytosis is quite common, especially among the pigmented cells. Sometimes a large pigmented cell is seen inclosing a smaller cell of the same type.

There is adjacent an evidently similar nodule which has been filled with hemorrhage. In this can be recognized the outlines of vessels and necrotic tissue and a great deal of brown pigment and pigment cells. With the connective-tissue stain the blood vessels are very clearly shown running everywhere through the nodule and closely surrounded by partly necrotic masses of tumor cells.

The larger nodule from this groin shows the tubular type of growth. (Plate III., Figure 3.) The capsule of loose connective tissue has many large irregular spaces filled with blood corpuscles. The tumor is everywhere threaded with a network of blood vessels, mostly filled with blood corpuscles. These have a definite endothelium, and surrounding this in the larger vessels are one or more layers of long narrow adventitia cells. The tumor cells are closely arranged about these vessels, forming a mantle of many compact layers. In a few places along the side of the tumor nodule the cells form a loose structure somewhat more compact close to the blood vessels, and midway between these vessels there are numerous irregular splits in the tissue. A little further from the wall these splits are larger and most often filled with blood corpuscles and fibrin, while the

tumor cells clustering about the blood vessels form more definite cylinders. Nearer the center there is between these cell cylinders more fibrin and necrosis of the intertubular cells, and in places definite fibers of connective tissue partly enclose the masses of cells about the blood vessels. In the very center the tubular character is much more marked. The blood vessel is generally larger and the connective-tissue stain shows delicate fibers of connective tissue running out between the tumor cells around the blood vessels, in some even to the connective-tissue layer which wholly surrounds the cell mantle. Thus the greater part of the tumor is made up of definite blood vessels surrounded by eight to twenty layers of cells; the whole being enclosed by a complete layer of connective-tissue fibers, forming an almost round tube. Between these tubes there are blood corpuscles, fibrin, and some loose tumor cells.

The cells of this nodule have the same general character as those already described. They are all nearer a medium size and vary less in shape. The most of them are round and the few elongated ones have their long axis perpendicular to the vessel wall. The nuclei are large and round, and show very many typical mitotic figures Many of them are phagocytic. None of them are pigmented. There is no evidence of any intravascular proliferation of the endothēlium, and the endothelial cells all appear to be normal, and there are no transitional forms between them and the tumor cells.

The tissue removed from the right groin consists chiefly of one large nodule and some smaller ones. These are definitely encapsulated and do not seem to be invading adjacent tissues. The largest nodule has very nearly the same microscopical characteristics as the last nodule described in the other groin. Lining the loose connective-tissue capsule is a quite compact tissue of tumor cells with a definite stroma, separated here and there by small blood vessels running out from the side. A short distance from the wall and in places even beginning at the wall are slits in the tissue midway between these vessels. Sometimes the division is not complete, several of these perivascular cell masses being close together in one mass. In other places the slits are filled with blood, fibrin, and loose tumor cells, but there is little of this near the border. There is also correspondingly less connective tissue to form the outer border of these cell tubes, although the intercelluar fibrillæ of connective tissue extending out from the vascular wall are well developed. In the very center of the nodule almost all the tubules have a smooth outer wall of connective tissue.

One of the smallest nodules shows the tubular type of growth very beautifully. There is considerable hemorrhage between the tubules, and though at the side this has split the tissue up very irregularly, in the center there are very definite perivascular cell cylinders surrounded in places by several layers of connective tissue. In the intertubular spaces there is much hemorrhage and fibrin and delicate strands of connective tissue. These perivascular tubules, either partially or completely enclosed with connective tissue, are well shown in Plate III., Figure 3.

An encapsulated nodule a little over one centimeter in diameter shows

near the border a growth of much the same type as that of the medium-sized nodule of the other side, a loose tissue of multiform cells traversed by blood vessels and broken up by intervascular slits. There is little hemorrhage except in the very middle of the nodule where it fills the slightly larger intervascular slits. The nodule appears to be a very vascular sarcoma broken up by hemorrhage into the tissue. In the middle there are a few connective-tissue fibrillæ forming around the cell cylinders. Only one or two of these are completely surrounded, and the tubular character is only poorly shown. A small bit of lymphoid tissue near this is infiltrated with large spindle-cells running in bands in every direction. They have large vesicular nuclei which rarely show mitotic figures.

On May 15, 1901, the patient was readmitted to the hospital. Her husband stated that five days before entrance she began to complain of headache, became slack in her work and careless about her dress. Three days before she seemed dazed and did not feel able to get up. She appeared indifferent to the exposure of her person, and two days before entrance became delirious at night. The next morning her husband noticed that she had difficulty in using her left arm and walked with a limp as if her left leg were affected. She seemed drowsy and continued to complain of headache. He stated that she had twitching of the left arm and hand two days before. She vomited the day before entrance and on the day of entrance. On examination she was found to be stupid and drowsy, but replied to questions. The pupils reacted equally, but the right was larger than the left. The left pupil reacted sluggishly to light. The tongue was protruded in the median line. There was no facial paralysis. She moved her right extremities rather than the left. The abdomen showed some induration about the scar of the old operation in the right groin. There was no evidence of recurrence of the tumor about the vulva. The left knee jerk was more marked than the right. Babinski reaction present on left. No clonus. No loss of sensation. On September sixteenth she was in coma. The left pupil was larger than the right. Both reacted equally. There was slight irregular twitching lasting half a minute, affecting the right hand and wrist and extending to the right leg. The pulse was increasing in rapidity.

Diagnosis of cerebral tumor involving the motor area of right side was made, and Dr. John C. Munro operated, trephining over the middle center of the motor area of the right side. The dura was under high tension and did not pulsate. On opening the dura there was very marked bulging of the brain. The pia was slightly opaque over the convolutions. There was no difference in the elasticity of the brain or in the feel. A director was passed towards the ventricle, no fluid escaping. As it was thought that the tumor might lie on the opposite side, a small trephine opening was made on the left and the ventricle tapped. Considerable serum escaped until the tension and pressure on the left became normal. The bulging brain on the right side did not recede on relief of pressure on left. A wedge-shaped piece was cut out of the convolution in approximately the region of the arm center, and close beneath the cortex was found a soft black sarcomatous-like tissue, fairly differentiated from the surrounding brain and extending upward toward the leg center. This growth was curetted out, leaving a cavity about the size of an English walnut, allowing the surface to reach the normal level. The wound on the left was closed with a capillary drain; that on the right was closed without replacing the dura. The patient required moderate stimulation, but was in fair condition at the close of the operation. She never recovered consciousness, however, and died two days after the operation.

The following is an extract from the autopsy protocol in this case:

Peritoneal cavity is normal. In the central part of the transverse mesocolon, very close to the colon wall, is a small dark colored nodule one-half centimeter in diameter. In the mesentery near its attachment to the ileum are two black nodules. One is situated twenty-eight centimeters from the ileo-cecal valve and is one centimeter in diameter and smooth in contour. The other, one hundred and fifty centimeters from the valve, is two centimeters in diameter and nodular. Both are quite firm on pressure. In the right groin just internal to the crural arch is a mass of three glands. The mass measures seven by three by two and one-half centimeters. When the capsule of the gland is cut much fluid blood and stringy material wells out. This is soft, dark red, and tears in long threads which

under the microscope are seen to be blood vessels surrounded by several layers of cells. Along the internal iliac artery is a similar gland about two centimeters long, and one-half to three-fourths of a centimeter in diameter. Above this near the common iliac is a smaller one, and in the retroperitoneal tissue below the right kidney and a little to the right of the inferior vena cava is a gland about the size of a pigeon's egg. All are of the same dark red color.

Lungs: Over the surface of both lungs are a number of black areas one-half to one centimeter in diameter, of irregular contour, and on section extending two to five millimeters into the lung. These are firm. They seem limited to the surface. On section the lungs are red, moist, and everywhere air-containing.

Uterus contains a dozen or more fibro-myomata from one to six centimeters in diameter, the whole making a nodular mass thirteen by eight by six centimeters. On section these are grayish-white, elastic, and easily peel out. In the centre of one is a soft brownish-red area, one centimeter in diameter.

Brain-weight 1,255 grammes; the right hemisphere bulges anteriorly and laterally at least one centimeter beyond the corresponding extent of the left hemisphere. The right prefontal area shows flattened convolutions with fluctuating area having a firm center, bluish-black in color. A distance of two centimeters inward and forward of the foremost point of the large trephine-hole, there is a dark blue-black area three centimeters in diameter; six centimeters from anterior pole of left hemisphere and four centimeters from the great fissure is another subpial nodule of similar aspect, one-half centimeter in diameter. The left occipital lobe shows a nodule one centimeter in diameter, two centimeters from the posterior pole. On the posterior-superior lobe of the right cerebellar hemisphere is a nodule one and one-half centimeters in diameter.

The middle portion of the right optic thalamus shows a nodule two by two by one centimeter, with major axis anteroposterior. These nodules on section resemble each other in having a firm, gray, red center, granular and somewhat thready, surrounded by a jelly-like, dark-red zone, with smooth section surface, fading rather suddenly out into normal brain tissue.

The brain, aside from the nodules, seems normal.

Anatomical Diagnosis. — Perivascular angio-sarcoma of deep inguinal and retroperitoneal lymph nodes with metastases in mesenteric lymph nodes, brain, and in fibromyoma of uterus.

The sections of the main tumor removed at autopsy show that these long cell cylinders, curiously interwoven, fill the entire cystic space. The intertubular blood and fibrin has washed out in the first hardening process. These cylinders or tubules have a definite outer wall of connective tissue. Some of them are a millimeter or more in diameter, and have a thick layer of connective tissue between the endothelium and the tumor cells and an outer covering of several layers of connective tissue. Lining the capsule there is a little sarcomatous tissue of a very vascular type that is irregularly divided by narrow spaces.

One of the deep inguinal lymph nodes shows an extremely vascular growth. (See Plate III., Figure 4.) The vessels are numerous and each one is surrounded by a mantle of tumor cells which in some parts is very scanty. The vessels appear to be so numerous that there are hardly enough tumor cells to go around. All these tubules have an outer coat of connective tissue and fibrin, which is rather thick and encloses some leucocytes and small round cells in its meshes. (See Plate IV., Figure 5.) There is considerable hemorrhage between the tubules, and in places there is thrombosis of the vessels and necrosis of all the tissue.

An external iliac lymph node shows a quite vascular growth surrounded by a dense capsule. There is considerable hemorrhage, which breaks up the tumor tissue very irregularly except in one part, where the tubules are evenly divided by masses of blood corpuscles. (Plate IV., Figure 6.) The cells of this nodule seem to bear a much more intimate relation to the blood vessel wall than those in the young metastases previously described. The endothelium of the vessels is everywhere normal.

Separated from the adjacent tumor growth by a thick fibrous wall is a large vessel lying in a space partly filled by hemorrhage. This vessel is surrounded, like the tubules of the main tumor, by a thick wall of tumor cells and an outer coat of fibrin and connective tissue.

Nodules from near the left inferior vena cava, and the large and small nodules on the ileum and colon show a very similar growth. (Plate IV., Figures 7 and 8.) There is a definite capsule surrounding a loose vascular tissue of the same structure throughout. Most of the cells are closely arranged about the blood vessels. There is more or less hemorrhage and loose tumor cells in the intervascular tissue, and in places there are leucocytes and round cells and necrosis. The tumor cells vary extremely in size, but are mostly round. The nuclei stain darkly and show a moderate number of mitoses. There are very many multinuclear giant cells and phagocytic cells. There are but few cells about each vessel, but these seem to be intimately associated with the adventitia. The endothelium of the blood vessel does not show any alteration.

A section of the soft brown spot described in one of the fibromyomata of the uterus shows a growth which suggests the alveolar type where it is invading the adjacent tissue, but nearer the center is quite vascular and split up by much hemorrhage, while the cells show a slight tendency to perivascular arrangement. The cells are mostly large, irregular in shape, and contain one or several darkly stained nuclei. The typical tissue of the surrounding fibro-myoma shows large areas of hyaline degeneration.

The metastases in the brain show a growth which is very vascular and somewhat edematous. There are more polymorphonuclear leucocytes and round cells and necroses in the intervascular tissue, and in places the perivascular growth is well marked. The nodules are small and there is no distinct tubular character. There are few giant cells and not many mitoses. The cells are not quite as much a part of the adventitia of the vessel wall as in the nodule on the intestine. The blood vessels of these nodules are occasionally dilated, giving a telangiectatic appearance.

The section of the lung shows a nearly normal tissue with occasional small areas where the capillaries are filled with blood and the alveoli nearly full of polymorphonuclear leucocytes, lymphocytes, and endothelial cells. There are a few phagocytic cells and almost no fibrin. A section through one of the pigmented areas in the pleura shows a small portion very definitely marked off by a thick fibrous wall. This area is extremely vascular, and there is considerable connective tissue throughout it. Along the border are small groups of lymphoid cells, but it is mostly made up of large irregular pigmented cells. In the connective tissue about this nodule the pigment cells are long and spindle-shaped with a long oval nucleus; in the centre they are larger, quite irregular, and have very darkly stained nearly round nuclei. They appear more like altered endothelial cells. The pigment is in large and small black granules, and does not generally fill the cell. It appears more like the anthracotic pigment usually found in the lung. Some of these cells are phagocytic. These cells have no definite arrangement, but are scattered about through this nodule. There are no mitotic figures. In sections of these lung nodules preserved in alcohol and stained for iron, the pigment granules appear perfectly black.

CASE II. — This second case is added from the pathological records because it is of the same type as the others and has taken a similar course.

C. M., a man sixty-eight years old, was seen in the Surgical Out-Patient Department April 30, 1896. He said that a short time before entrance he had first noticed a warty growth on the glans penis. "Some one told him to tie a horse hair lightly around it and it would go away." The top of the wart was gone, but the glans was inflamed. For one or two years he has been rising at night to micturate.

Microscopic examination. — A small portion of the nodule on section shows a tumor situated superficially; the epidermis over it is not ulcerated. In a large part of the tumor there is hemorrhage. The tumor is composed of several separate masses. Between them there is rather loose tissue with a great deal of hemorrhage in places, with numerous blood vessels and spindle-shaped cells. In some of the tumor masses there are also large vascular spaces. With a high power the tumor is seen to be composed of large, irregular, and spindle-shaped cells. In the looser parts there is considerable hemorrhage. There are numerous blood vessels with very indefinite walls.

He was admitted to the hospital June 29, 1896, but operation was not then advised. November 2, 1896, he was again

admitted to the hospital. Since last June he has been passing bloody urine all the time, and has had one hemorrhage of "nearly a pint." Physical examination showed a rather emaciated old man. The growth in the penis has not increased much in size. There could be felt, however, a distinct line of thick induration running up the dorsum of the penis. The inguinal lymph nodes on the right side were somewhat enlarged and very hard. A portion of the penis was amputated by Dr. J. C. Munro.

The gross examination was as follows:

On slitting up the glans there is a tumor mass which projects within the urethra. This mass is three and one-half centimeters long by one and one-half centimeters thick. The mass is attached to the lower surface of the urethra over an area one and one-half centimeters long by one-half centimeter broad. The mass when hardened is of a grayish color; when fresh it was comparatively firm. The cut surface is almost homogeneous, but shows a very few irregular fissures. The anterior extremity projected to the meatus. The very tip projected through, and is necrotic. The general shape is that of a pecan nut. The surface is smooth. The urethra is dilated posterior to the growth. No induration of the tissue in this vicinity.

Microscopical examination. — Sections were made directly through the growth and stained with eosin and hematoxylin.

The mucous membrane of the urethra immediately adjoining the growth is somewhat thickened and infiltrated with round and epithelioid cells. At the origin of the tumor from the urethra it comes in contact with this submucous epithelioid infiltration. The tumor cells are immediately in contact with this, but they do not appear to pass into it, though the epithelioid infiltration at this point so closely resembles some of the cells of the tumor that it is difficult to say they are not the same. Over the surface of the tumor, for almost its entire extent, there is a very thin epithelial covering. In places this is perfectly definite; in places only traces of it can be seen, and in still other places it is entirely absent. There is some hemorrhage and necrosis on the surface of the tumor. The tumor is a *sarcoma*. In its deeper portions there are large masses of cells, spindle and multiform, without any very definite arrangement, and containing numbers of nuclear figures. The large masses of cells are separated from one another to some extent by fissures, and there are smaller fissures proceeding off from these. There are also numerous small openings and splits in the tumor, apparently not connected with the larger fissures. These smaller splits apparently correspond to blood vessels and contain blood corpuscles. In other parts of the tumor this appearance is better marked. There is a general division of the tumor here into lobules, and

in the center of the lobules a blood vessel is found. In still other parts of the tumor there is an arrangement somewhat suggesting an epithelial growth, the cells along the sides of the vessels being arranged more or less in single masses and growing with much irregularity. Among the cells there are many very large ones with several nuclei. In its general arrangement it has a certain amount of similarity to the angio-sarcomata, and it probably develops from the adventitia of the blood vessels. The spaces which are found in the tumor may be considered as lymphatic spaces. There is a small portion of the glans in which the epithelium is somewhat thickened, and in the subepithelial tissue there is the same intense infiltration with epithelioid cells, which very closely resemble tumor cells, and in places this growth appears to be breaking into the lower limits of the epithelium.

He was readmitted to the hospital November 25, 1898. Several months before this he began to notice a swelling in the right groin; gradually increasing in size, it was now about the size of a hen's egg. Two months ago the growth began to recur in the stump of the penis.

In the right groin a tumor about eight centimeters in diameter could be felt. It was freely movable. On the indurated stump of the penis were several nodular growths projecting from beneath the foreskin. The inguinal lymph nodes and part of the penis were removed.

The specimen is in two parts. One is the amputated portion of penis, about four and one-half centimeters long and four centimeters thick, covered with skin. At the distal end is a raised, cauliflower growth white to purplish-black in color, about one and three-fourths by one centimeter and of firm consistency. At the base of the growth are two old cicatrices. The other part of the specimen is an egg-shaped mottled mass four centimeters long by three centimeters thick, of firm consistency. On section the mass appears mottled-gray and reddish-brown. The surface is covered with a thin blood-stained fluid.

Sections from the recurrence at the original site show a growth of the sarcoma type which is everywhere invading the surrounding tissue. Near the border and scattered through the tumor there are fairly well-defined alveoli each containing a few tumor cells and separated from one another by a fair amount of stroma. The alveolar arrangement is lost in the greater part of the growth, and the tissue is broken up by very irregular spaces and the tumor cells grouped about the blood vessels. There are many which have a very scanty endothelial wall, and there are some round spaces containing blood corpuscles, which have a fairly definite border apparently formed by the stroma and tumor cells without any definite en-

dothelial lining. The tumor cells vary much in size and shape, but are mostly fairly large rounded cells. They have large round nuclei in which there are many chromatin granules and fairly numerous mitoses. There are some multinucleated cells. None of these cells are pigmented, although there is some light brown pigmentation of some of the cells in the stroma and surrounding tissue.

The tumor from the groin is mostly enclosed by a fibrous capsule which in places is much infiltrated with lymphocytes. The cells vary much in type. Most are fairly large round cells with a large nucleus, generally rich in chromatin granules. Many of the nuclei stain very darkly and show various stages of karyokinesis. There are a great number of large giant cells with five or six nuclei; some of these are phagocytic. There are a few places in the border in which a little lymphoid tissue remains. The tumor cells are invading this tissue through the lymph spaces and vessels and in places there are round masses and long columns of them. There is considerable congestion and hemorrhage into this lymphoid tissue. The most of the tumor is a pretty solid growth traversed here and there by definite bands of connective tissue and blood vessels. There is considerable stroma between the cells and some lymphocytes in it. In parts of the tumor the cells are very closely arranged in several layers around the blood vessels, and midway between the vessels the tissue is split and in this space there is sometimes blood, fibrin, necrotic material, and occasional lymphocytes. The tissue shows a very typical perivascular tubular arrangement.

The sarcoma according to Virchow in 1860 was considered to be a connective-tissue tumor in which the cellular elements predominate. The latest definition, that of Ribbert (1901), differs very little from the earlier ideas. He says it is a connective-tissue tumor with extensive development of the rich protoplasmic cells. Paget, Lancereaux, and Cornil and Ranvier classed the sarcomatous tumors as forms of fibroma.

In 1858 Billroth gave the name cylindroma to an interesting type of sarcoma. This name through common usage has been retained up to the present time, although such a name has no place in the true classification of tumors. We find that a cylindroma was defined as

> a tumor with cylinders and rounded structures having a hyaline character and mucous-like tissue. In their center is often a blood vessel with hyaline mantle, and between these are masses of tumor cells. The cylinders arise from hyaline or mucous degeneration of the vessel walls.

The later work of Billroth used the terms alveolar and plexiform sarcoma for these characteristic tumors which Waldeyer called angiosarcomata from their histogenesis. He recognized their origin from the blood vessel walls. Virchow has cited two cases of alveolar and two of plexiform sarcomata. He expressed much doubt as to the origin of these tumors, and was disposed to believe that they were of a mixed sarcomatous and carcinomatous type. Rindfleisch, in 1873, thought the alveolar sarcoma to be a carcinomatous degeneration of sarcoma, and called it a sarcoma carcinomatodes. Other names have been used to designate the tumors of this group; as the Siphonoma of Henle and Brush, Schlauchgeschwülste, Schleimcancroid, Lymphangio-carcinosarcoma intravascularis, and some still more varied.

There has always been much discussion as to the type of this tumor. Even now, in the latest books of v. Hansemann and Ribbert, there is much doubt expressed as to the place of the alveolar sarcoma, and the question is raised whether it should not be struck out of the group of sarcoma.

The name endothelioma was first applied by Golgi in 1869 to designate a tumor arising from the cells of the smallest capillaries. In 1874 Sattler explained that this type of tumor was the product of pathological hyperplasia of the endothelium, and that the hyaline degeneration from which the term cylindroma arose is not a necessary characteristic of endothelial tumors.

Four years later Kolaczek collected from the literature the tumors of this kind which had hitherto been described under various names. He agreed with others in the belief that they arose from the endothelial proliferation of the blood vessel walls and only rarely have the lymph vessels any part in their origin. In his second article on angiosarcoma, indeed, he has to use some artificiality to include some of the eight cases he reports under this category. Many writers believe that Kolaczek has misplaced the matrix from which these tumors arise. Ackermann and Neumann assert that they rarely arose thus from the intima, but more commonly from the endothelium of the intervascular lymph spaces. Von

Hippel and Barth distinguish as of equal importance both the groups of hemangio- and lymphangio-sarcoma.

Eberth, in 1870, was the originator of the term perithelium to apply to "the highly developed flat layer of endothelial cells" which forms an outer covering for the blood vessels of the brain and spinal cord. Among others who have studied the perithelium, Kolaczek recognized special adventitia cells in a great number of capillaries, and Sortoli, v. Luschka, v. Brunn, and others have described a so-called perithelium in the pia-mater, testicle, and coccygeal, carotid, pineal, breast, and parotid glands. Waldeyer has described the perithelium in the vessels of the testicle as "a thick, rich layer of cells not always sharply marked off from the surrounding tissue, and appearing more often to run into the spindle-shaped bodies of the connective tissue." But the word from its derivation must mean a lining membrane, and it seems to be best defined by Driessen, Perthes, Hildebrand, and Best, who conceive it to be the lining endothelium of the perivascular lymph spaces.

Such is the earlier history of the controversy as to the origin of this group of tumors. Virchow says, not the histological picture of a tumor or the type of its cells, but the manner of its origin and the tissue from which it springs must decide the classification of tumors.

Ackermann states that all sarcomata have blood vessels as their mother tissue. This is, however, probably far from correct. At least the vessels of sarcoma are much more numerous than those of any other connective-tissue tumor. The angiosarcoma is an angioma with sarcomatous proliferation of the vessel walls. Lucken believes that the development of vessels is primary and chief and the sarcomatous proliferation of blood-vessel cells is secondary, and calls it an angioma sarcomatosum. Thoma calls them cellular varieties of hemangioma and distinguishes two forms, telangiectatic sarcoma and perithelial sarcoma, and both may be combined in the same tumor.

According to most writers these tumors may arise from

either the endothelium of the blood vessel or that of the perivascular lymph space — that is, the perithelium. Many cases of simple endothelioma have been described. Tumors of the perithelial type have been reported by Putiata, Waldeyer, Jaffes, v. Hippel, Barth, Manasse, and others. And some of the cases of Driessen, Hildebrand, Paltauf, Paoli, Perthes, v. Rostkorn, and Best show tumors arising at the same time from both the endothelium and perithelium.

Volkmann makes no principal distinction between the perivascular sarcoma and the endothelioma, but describes the adventitial proliferation as a "secondary and quantitatively insignificant process" in the tumor from the endothelium. In the great material at his disposal he has only once seen a growth exclusively out of the capillary wall proliferations, but finds it combined with lymph-endothelium proliferation. Malherbe in his recent study of sarcoma considers the angiosarcoma as of endothelial origin alone.

It will be seen that many varieties of this type of tumor have been described, but there has been no satisfactory attempt to classify them. Manasse in a recent article seems to offer the best arrangement. Besides the two forms of blood and lymph vessel endothelioma, he includes with the angiosarcoma a third variety, the perivascular sarcoma. He says it arises from proliferation of the adventitia cells. He describes it as a very spongy mass of vessels, both parallel and crossing one another, and each surrounded by one or more layers of polyhedral, cuboidal, cylindrical, or elongated cells. The greater part of the tumor consists of vessels with only a thin normal endothelium, and a thick perivascular mantle of spindle-shaped epithelioid cells containing many mitotic figures. Most of the cases of Waldeyer, Putiata, and Kolaczek, and a few of the kidney tumors of Paoli and the three cases of Maurer come under this description. Manasse says this type of tumor is from the adventitia cells, whether they are called perithelium or not, and is not a lymph-vessel endothelioma.

Recently Borrmann has still further divided the angioma. After mentioning the simple angiomata, hemangioma and

lymphangioma, he considers the others as endotheliomata from the blood vessels, the lymph vessels, or the capillaries, and adds two other varieties of unknown origin. The perithelioma consists principally of vessels, around the outer wall of which are several layers of cells, with their long axis perpendicular to the vessel wall, and the periendothelioma is similar except that the cells have their long axis parallel to the direction of the vessels. The cells in the perithelioma are like those of the spindle-cell sarcoma, are arranged radiating from the vessels, are the only structure between the vessels, and in no way related to the endothelium. In periendothelioma, a case of which has just been reported by Wells, the cells are parallel to the wall in marked contradistinction to the perithelioma, but this type does not seem to be well differentiated from the lymph-vessel endotheliomata, which are not uncommon, especially in the brain. This perithelioma is evidently the same as the perivascular sarcoma of Manasse. Now the term perithelioma, as Borrmann here uses it, is not well chosen, for most observers agree that the perithelium is the endothelial lining of the perivascular lymph space, and he calls the tumors arising from those cells the lymphoangio-endothelioma.

It is simpler to adopt the terminology of Manasse, which includes all the forms that Borrmann names, and is satisfactory, at least as far as it goes. Thus we have the hemangio- and lymphangio-endotheliomata and the perivascular sarcoma. It is to the third group that the two cases here reported belong.

The reasons for classing these tumors as perivascular angiosarcomata will be best shown in a review of the principal points in their development. In the first case the original growth was a melano-sarcoma developing in a mole. Formerly the pigmented mole was thought to be derived from any place in the connective tissue and distinguished only by its pigment. V. Recklinghausen was the first to assert that pigmented nevi and the melanosarcomata arising from them originate from the endothelium of the lymph spaces. Unna thought they originated from bits of epithelial tissue which

have previously been included and surrounded by connective tissue. The latest and most generally accepted view is Ribbert's modification of the older idea, in which he claims that they develop from special pigment-producing connective-tissue cells. However we may explain the origin of this tumor, its histology and subsequent development is surely that of a connective-tissue growth. The spindle-shaped cells, the mitoses and the growing border of this first nodule are all suggestive of a malignant tumor.

The pigmentation of this tumor is of the true melanotic type. The granules are mostly within the cells, and in the newer cells are much lighter and smaller. The pigment cells show the chief types described by Ribbert in his work on melanosarcoma. In the primary growth and in the small nodule of the recurrence we find the pigment mostly in the surrounding connective tissue and especially thick in those cells lying in the adjacent skin. Possibly this is suggestive of its origin in the normal pigment-producing cells. It is noticeable that the pigment in the recurrence is also most marked in the stroma and in that part of the tumor which has more the character of a spindle-cell sarcoma. In the metastases in the groin we find in the earliest nodules that the invasion consists almost wholly of pigment cells. This agrees well with the view that the pigmented sarcoma is the most malignant type. In all the pigmented metastatic growths, the invasion was by means of the lymph channels. In those metastases which were dependent on the blood current for their origin we find no pigment cells and the light brown color is entirely due to intercellular hemorrhage. This, however, may be explained by the fact that all the true pigmented nodules developed by direct metastasis from the pigmented tumor of the skin, while the others were only indirectly from them. We know that in the generalization of a melanosarcoma of the skin the metastatic nodules are nearly always pigmented and generally more deeply than the original growth. It may be that the cells carried from the secondary nodules to the tertiary group of metastases were not pigment-producing cells.

In the recurrence at the primary site we find a very interesting growth in which the greater part closely resembles a carcinoma. (Plate III., Figure 2.) This type of alveolar sarcoma represents the mixed carcinomatous and sarcomatous tumors of many writers. This appearance probably occurs where the formation of cells is very rapid and there is at the invading border of the growth a nearly typical alveolar arrangement. With its larger cells and many more mitoses the alveolar part of this nodule shows much more evidence of rapid growth than the rest of the tumor. According to Stengel the sarcomata springing from moles or warts give rise to the alveolar forms.

Metastases may be carried by the blood vessels or the lymph spaces about the capillaries. Sarcomata often extend to the neighboring tissues by rows of cells following along and around the vessels. Sarcomatous cells in the very vascular nodules are only separated from the blood current by a thin layer of endothelium which is often ruptured; thus the infectious cells or bodies of the tumor reach the blood current. In our case all these methods prevailed. The invasion of the lymph nodes in both groins was through the lymph channels. In the earliest metastases we find the tumor cells in the lymph spaces both inside and around the lymph nodules. The external iliac lymph node on the right was probably infected by direct extension along the vessel wall, which the large isolated vessel near it shows. The tumor was carried to the ileum, the colon, and the brain by the blood current. The fact that the lungs were not infected is a very interesting point, although the tumor cells must have passed through the lungs in order to reach the brain. The lungs are the most common seat of melanotic metastases through the blood. The pigmented nodules on their surface, however, show no evidence of tumor cells. The change of type of this tumor from a melanotic spindle-cell sarcoma to a tubular perivascular sarcoma is most remarkable. Thoma is almost the only one who makes mention of the fact that perithelial (as Thoma terms it) sarcoma may in places form a transition stage in spindle and round

cell sarcomata which show no unusual vascularity. The first sign of this that we noticed was in the second lymph node from the left groin. In this have been described round spaces without any endothelial lining which were bordered by tumor cells. Rarely a few blood corpuscles were seen in them. Similar spaces were noticed in the next nodule described and their resemblance to the vaso-formative cells of the angioplastic sarcoma was noted. These do not have the appearance of being fat drops and they may represent the origin of the new blood vessels of the tumor. In the next nodule described there is seen the transition stage, a loose cellular growth with no definite arrangement; the cells are more or less arranged along the blood vessels and the whole tissue is everywhere broken up by narrow slits. This peri-vascular disposition of the cells was a little more clearly shown in a hemorrhagic nodule near by, and was also very well marked in part of the recurring growth at the primary site of the second case. We see here simply a very vascular growth in which the cells are to some extent clinging about the new formed blood vessels. In the second nodule from the right groin the next stage of this change is shown. Here the cells have increased very rapidly and fill most of the intervascular spaces. It is a simple spindle-cell sarcoma; here and there hemorrhage has occurred and the cells being clustered about the blood vessels for the sake of nourishment, the extravasated blood has filled the tissue between the vessels. With the increase of hemorrhage the tissue may be broken up, leaving numerous cylinders of cells surrounding blood vessels and lying in a mass of blood corpuscles. (Plate IV., Figure 6.) There is extending out from the vessel wall an intricate network of connective-tissue fibrillæ between the surrounding cells which serves to hold the cylinders together. Later the extravasated blood degenerates, but no evidence of organization was seen. Fibrin is formed and delicate connective-tissue fibrillæ either extend out from the vessel wall or develop in the surrounding masses and surround the cell cylinders. Thus is formed an outer wall of connective tissue which later becomes connected with

the inner vessel wall by delicate strands of connective tissue. (Plate IV., Figure 5.) In the smaller nodules where hemorrhage has occurred this takes place very early. In most of the larger nodules where there is no hemorrhage the outer wall of these tubules may not be developed. This is especially so in the second case where the growth in the groin was a firm tumor with very little hemorrhage, and it shows a typical perivascular arrangement only in places. In some growths where there has been little hemorrhage there is often a rapid cell proliferation, and many tumor cells lie in the intertubular spaces surrounded by blood corpuscles, fibrin, and lymphocytes. This has been noted in the cases of Paoli and Driessen. Necrosis often occurs here probably from the lack of nutrition, and this aids in the development of the connective tissue and the formation of tubules. In the oldest growth, the large one in the right groin, we see the typical tubular type. It is a large tumor with definite capsule, and there has been considerable hemorrhage and necrosis. The entire growth has been broken up, leaving around the wall a very little sarcomatous tissue which does not show this tubular character. The whole cyst is filled with cell cylinders lying in a mass of blood and necrotic material. The cell cylinders have a definite smooth outer wall of connective tissue. (Plates III. and IV., Figures 4 and 5.) There is no evidence of the hyalin degeneration which often occurs in the cell mantle or the endothelial wall of these tumors.

The nodules of the intestine and in the brain show very young metastases where probably cells brought by the blood current have broken through and developed in the vessel wall or the perivascular lymph spaces. These cells show the same type as those of the original growth. They are in very intimate relation to the blood vessel wall, but in the examination of very many sections no evidence of any proliferation of the endothelial wall was seen, and there was no sign of any change in the type of the endothelium of the cells. The large size and irregular shape of some of these tumor cells close to the endothelium does not in the least signify that they are proliferating from the endothelium; they are simply

sarcoma cells that are well nourished and better developed. (Plate IV., Figures 7 and 8.) Malherbe has noted this same fact in carcinoma. In describing spindle-cell sarcoma, Malherbe says: "Where the cellular proliferation takes place along the vessels we have the perivascular variety of sarcoma. Here the cells close to one another have a luxuriant growth around the vessels as the grass of the prairies along the banks." So this tumor does not seem to be a perithelioma or a perithelial angiosarcoma, but rather a tubular perivascular sarcoma.

PROGNOSIS. — The general course of these tumors is that a pigmented growth of long duration suddenly develops rapidly, and after incomplete removal there is a recurrence at the site of operation, and not very much later generalization of the growth, cachexia, and death. If the metastatic tumors be situated near the surface, or perhaps on the mesentery, the tumor may develop slowly. Hemorrhage may cause a rapid increase in the size of the tumor which then becomes fluctuant. The pressure of the hemorrhage causes new degeneration. New vessel walls are weakened and new hemorrhage follows. Thus a hemorrhagic cyst is formed. In the first case the excision of the primary growth should have included the whole area of pigmented skin. The fact that a second small nodule similar to the primary growth was found near the recurrence at the site of the first operation shows that the tissue was infected .beyond the limits of the first excision. The fact that at autopsy the so-called pigmented mole removed from the vulva shows a sutured wound with no evidence of recurrence suggests that the second operation was locally successful. The surgeon should go well beyond the limits of the growth even at the risk of mutilation. It is asserted that the removal of a small pigmented growth often results in the rapid dissemination of the tumor. Van Werts raises the question as to whether the chance of rapid recurrence and metastases is not increased by the operation of removal. We will say that this is probably not so. Whether there is any importance in the fact that tumor cells might be

found in the blood during the generalization of malignant tumors or not, it would at least be an interesting study to make on a suspected case. As an aid to diagnosis the value of this process is doubtful.

On reviewing the history in connection with the pathological findings the chief conclusions to be drawn from these cases are as follows:

1. A pigmented growth of the skin may at any time take on malignant characteristics.

2. If the removal of a pigmented growth be undertaken at all, it becomes important for the surgeon to cut wide of the growth, as is the rule in carcinoma. All pigmented tissue should be removed practically in one piece, and without cutting through the tumor tissue.

3. Metastasis may take place from the peripheral organs to the brain, without the lungs being involved.

4. A melanotic sarcoma of the skin may in its metastases assume the type of a perivascular angiosarcoma.

REFERENCES.

Mallory. Staining Methods, 1900, Jour. of Exp. Med., Vol. v., No. 1.

Mallassez et Monod. Arch. de physiologie, 1878, p. 375.

Cartnot et Marie. Sarcome angioplastique. Bull. soc. anat. de Paris, 1898, lxxiii., p. 82.

Virchow. Die Krankhaften Geschwülste, Bd. iii., S. 257.

Ribbert. Allegemeine Pathologie, 1901.

Billroth. Beiträge zur Geschwülstlehre, Arch. f. klin. Chir., 1859, Bd. xi., S. 230.

Waldeyer. Die Entwickelung der Carcinome, Virch. Arch., 1867, Bd. xli., u. lv.

Rindfleisch. Lehrbuch der pathol. Gewebelehre, 1873, p. 107.

v. Hansemann. Die mikroskopische Diagnose der bösartigen Geschwülste, 1897.

Golgi. Ref. Virch. Arch., 1870, Bd. li., p. 311.

Settler. Uber die sogen. Cylindrome, 1874. Cited by Tanaka.

Kolaczek. Ueber Angiosarkom, Deut. Zeit. f. Chir., Bd. ix., S. 1–48.

Kolaczek. Ueber achte neue Fälle, Deut. Zeit. f. Chir., Bd. xiii., S. 165.

Ackermann u. Neumann. Arch. f. Heilkunde, Bd. xii.

Neumann. Dermoid Cyste mit perithelialer Degeneration, Arch. f. Gyn., 1899, Bd. lviii., S. 185.

Von Hippel. Beitr. z. Casuistik des Angio-Sarkoms, Zieg. Beit., 1893, Bd. xiv., S. 370.

Barth. Ein Fall von Lymphangiosarkom, Zieg. Beit., Bd. xix., S. 462.

Eberth. Ueber die Blut- und Lymphgefässe des Gehirns, Virch. Arch., Bd. xlix., S. 48.

Sortol, v. Luschka, v. Brunn. Cited by Kolaczek.

Driessen. Untersuchungen über Endotheliome, Zieg. Beit., Bd. xii., S. 65.

Perthes. Bruns's Beit. z. klin. Chir., Bd. xii. Cited by Best.

Hildebrand. Ueber das tubulare Angiosarkom, Deut. Zeit. f. Chir., Bd. xxxi., S. 263.

Ackermann. Histogenese und Histologie der Sarkome, Volkmann's Vorträge, 1883, N. 233.

Lucken. Ueber Angiosarkom, Deut. med. Wochenschr., Leipzig, Bd. xxii., S. 1189.

Thoma. Lehrbuch der path. Anat., 1894, p. 701.

Putiata. Ueber Sarkom der Lymphdrüsen, Virch. Arch., Bd. lxix., S. 245.

Manasse. Zur Histologie der primaren Nierengeschwülste, Virch. Arch., Bd. cxliii., S. 323.

Paltauf. Ueber Geschwülste der Glandula carotica, Zieg. Beit., Bd. xi., S. 69.

Paoli. Beitrag z. Kenntniss der primären Angiosarkome der Niere, Zieg. Beit., Bd. viii., S. 140.

Rostkorn. Arch. f. Gyn., 1882, Bd. xli.

Best. Ueber Sarkom der Aderhaut, Zieg. Beit., Bd. xxxi., S. 253.

Volkmann. Deut. Zeitschr. f. Chir., 1895.

Malherbe. Recherches sur le Sarcome, Gaz. Med. de Nantes, 1898–1901.

Maurer. Ein Beitrag zur Kenntniss der Angiosarkom, Virch. Arch., Bd. lxxvii., S. 346.

Borrmann. Nomenclatur der Blutgetfässgeschwülste, Virch. Arch., Bd. clvii, S. 297.

Wells and Brower. Case of Periendothelioma, Am. Jour. Med. Sc., 1901, cxxii.

Unna. "Naevi carcinome," Berliner klin. Wochenschr., 1893, p. 14.

Ribbert. Ueber das Melanosarkom, Zieg. Beit., Bd. xxi., S. 471.

Thoma. Lehrbuch der pathol. Anat., 1894.

Van Werts. Sarcoma of Thigh and Inguinal Glands, Bull. soc. anat. de Paris, 1898.

Kauffmann. Lehrbuch der spec. pathol. Anat., Berlin, 1896, p. 779.

Tanaka. Ueber die klinische Diagnose von Endotheliomen, Deut. Zeit. f. Chir., 1899, Bd. li., S. 209.

Stengel. Text-book of Pathology, Philadelphia, 1898, p. 144.

DESCRIPTION OF PLATES III. AND IV.

(These all represent the first case.)

1.— Original tumor on labium. Hematoxylin and eosin. Magnification 35.

2.— Recurrence at primary site. Hemalum and eosin. Magnification 350. Showing alveolar structure and type of cells.

3.— Metastatic tumor in groin. Connective-tissue stain. Magnification 85. Showing typical tubular growth, and in places new formation of connective tissue.

4.— Deep inguinal lymph node. Hemalum and eosin. Magnification 45. Very vascular tubular growth.

5.— Deep inguinal lymph node. Hemalum and eosin. Magnification 150. Showing outer wall of typical cell tubule and the relation of tumor cells to the adventitia.

6.— External iliac lymph node. Connective-tissue stain. Magnification 150. Showing perivascular arrangement of tumor cells and hemorrhage between the tubules.

7 AND 8.— Small metastasis on ileum. Hemalum and eosin. Magnification 350. Showing intimate relation of tumor cells to the blood-vessel walls in the youngest metastases, the slight tendency to separate into tubules, and the intertubular hemorrhage.

FIG. 1.

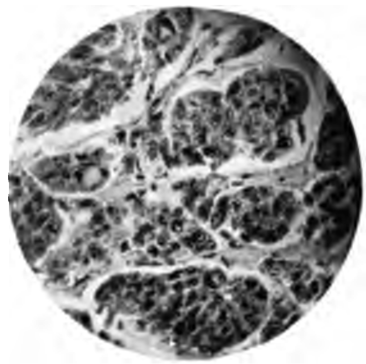


FIG. 2.

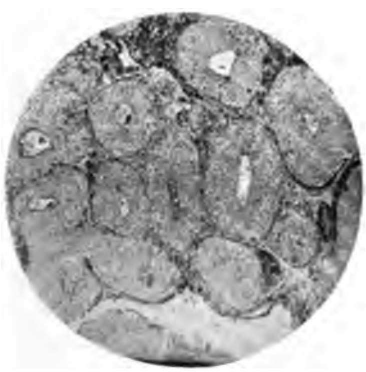


FIG. 3.

FIG. 4.

LOW AND LUND. TUBULAR PERIVASCULAR SARCOMA.

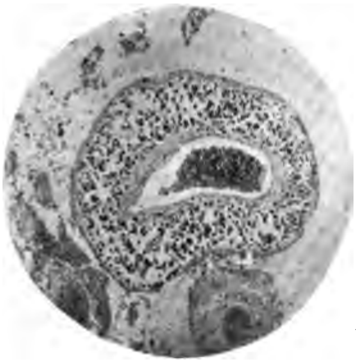


FIG. 5.

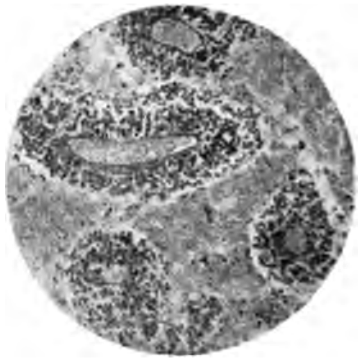
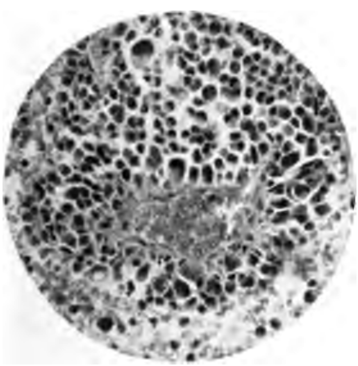
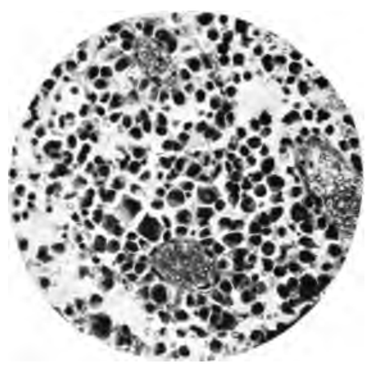


FIG. 6.





FIGS. 7 AND 8.

THE INTERCUNEIFORM BONE OF THE FOOT: A NEW BONE.

THOMAS DWIGHT.

It has been my fortune to have met with two instances of an occasional bone in the human foot which had apparently never been observed. I have moreover a tolerably distinct recollection of having seen it many years ago. If the dorsum of the foot be examined carefully, one almost invariably sees a little fossa made by the opposed surfaces of the internal and middle cuneiform bones. It is usually more at the expense of the second than of the first. It is between their proximal ends where they rest against the scafoid, and extends, perhaps, along the proximal half of the shorter middle cuneiform bone. Although this little pit has not been described, it has been figured in many works.

Case I. (Plate V. Fig. 1.) This specimen, which is the better one, comes from the right foot of a white man aged fifty-three; Warren Museum number 9390 a-6. The condition of the bones is practically normal. The *intercuneiform* occupies this little fossa. It is wedge-shaped, the thin edge being the deepest part. The length is eleven and one-half millimeters. The greatest breadth is four and one-half millimeters, and the greatest depth four millimeters. The proximal end is rounded, the distal one pointed. There is no articulation between this bone and the scafoid. Its relations in this respect with the cuneiforms are rather difficult to determine, but it seems possible that it was once joined by cartilage to the middle cuneiform. Its relation to that bone seems much more intimate than to the inner cuneiform. The little fossa occupied by this bone is no larger than is often observed.

Case II. (Plate V., Fig 2.) This is also the foot of a white man. His age was sixty. The Museum number is 9390 a-7. The first and second toes present some pathological, probably rheumatoid, outgrowths. The tarsal bones show no signs of disease, but the *intercuneiform* bone itself looks less normal, so to speak, than that of the first case. It is smaller, the length being eight and one-half millimeters, the

breadth five millimeters, and the depth four millimeters. It is less distinctly wedge-shaped and the dorsal surface rises more irregularly so as to resemble a quasi-accidental ossification. Like the other it shows signs of more intimate union with the second than with the first cuneiform.

This foot shows also a good specimen of the occasional ossification known as *calcaneus secundarius.*

As to the interpretation of the *intercuneiform* bone, I can only say that I know of nothing in comparative anatomy to give it any special significance. I incline to think it the result of a second center of ossification of the middle cuneiform.

Since I sent an account of it, substantially the same as the above, to the *Anatomischer Anzeiger* I have received a letter from Professor Pfitzner of Strassburg, who has earned the title to be the authority on variations of the carpus and tarsus. He had written me before that the bone is absolutely new; he now sends some photographs of nodules fused with the middle cuneiform which very certainly can be nothing but *intercuneiform* bones, though not free ones. One of the points which his researches have done much to make clear is that the same element may appear free, or fused with one of several neighboring bones, and that it may to some extent shift its position. I am of course very much gratified at these observations, which show two things, first that I seem to have found what may be called a real element of the foot, and secondly that I was correct in emphasizing its affinity for the middle cuneiform.

It may be asked what meaning is attached to the assertion that it is a *real* element of the foot. If it be true that for the ground plan of the hand and foot we must look to something like a paddle with at least seven rays and with several transverse tiers of carpal or tarsal bones, it is conceivable that a certain element, absolutely of no practical use, should appear on the scene only at very long intervals. The fact, however, that it presents itself in very nearly the same place whether fused or free speaks for its being something more than a mere accidental ossification; and so much may be claimed for the *intercuneiform.*

FIG. 1.

FIG. 2.

BRANCHING IN BACTERIA WITH SPECIAL REFERENCE TO B. DIPHTHERIÆ.

HIBBERT WINSLOW HILL, M.D.
(*Director; Boston Board of Health Bacteriological Laboratory.*)

The term "branching" is used in this article to mean apparent union between two more or less elongated bacterial bodies, or parts of bodies, such that one end of one body lies at any point in the length of the other, except an extremity. This definition is adopted to avoid any à priori implication as to the nature of such union, which the use of the term in its ordinary sense might seem to involve.

The chief problems of the subject divide themselves into those of the nature of the branching, the actual morphological mechanism; and those of the origin of the branching, the physiological or developmental relationships. Both are very complex. To place the whole matter of branching as it occurs in different species on a definite basis is as yet impossible. It is not safe to reason closely from one species to another nor can the branching even in one species be regarded as yet well worked out. In this article, attention is confined to the diphtheria bacillus and the results are suggestive rather than satisfactory.

The writer believes the general subject one of fundamental biological importance to bacteriology and, as it affects the diphtheria bacillus, of considerable practical importance in public health work. Indeed theory and practice are more intimately interwoven in hygienic bacteriological work than in any other department of medicine. An intimate knowledge of the morphology and biology of the diphtheria bacillus is peculiarly requisite to meet the demands of every-day work in the diagnosis and release of diphtheria cases. To the failure to recognize this is due some at least of the difficulties met with in practice.

The writer, interested in branching diphtheria since 1895,

has of late been more strongly impressed with the importance of the whole subject. Wesbrook [10] in 1899 made public the results of a laborious and painstaking investigation into the morphology of the diphtheria bacillus, passing over the branching forms it is true, but placing the various morphological types encountered in cultures on the most definite basis yet set forth. That his morphological types, purely as such, do exist, no one familiar with diphtheria diagnosis can deny. What the relationships of these types may be to each other, whether or no the types are directly convertible, how they are related, if at all, to clinical types of the disease, to toxicity, to virulence, and to the distribution of the bacillus in nature are problems not yet on a satisfactory basis. Much attention has been paid to dried and stained preparations from cultures in these studies, but the information thus gained must always be largely inferential, although its aid is indispensable. Recognizing this, the writer has studied the morphology and development of individual diphtheria bacilli directly under the microscope. Although attention was given largely to the study of the relationships of Wesbrook's types, branching bacilli, when encountered, were carefully observed also.

Elsewhere [18] the writer's technique is described in detail. Here only the rather sparse results so far achieved connected with the branching itself are dealt with.

The principal hypotheses relating to the nature of the branching of B. diphtheriæ which have been offered up to date are:

Hypothesis one: That the branching is apparent only, due to accidental apposition, and merely an optical illusion.

Hypothesis two: That the single unbranched bacillus, whatever its size, is a single-celled organism; that the branch arises by a pushing out at one side of the cell contents; that this continues until the projection assumes more or less the general size and shape of the original mother rod; constriction at the base of the daughter rod may follow until it separates entirely; or without separation either rod may

again branch, giving rise to complicated figures; or either rod may divide further by fission only. (See Figs. 9 to 15, 27 to 30.)

Hypothesis three: That the bacilli are single-celled rods only when very small; that the large (often clubbed) rods, in cultures of which branching seems to be most often found, are really short chains of cells with little or no interval between adjacent members; that these rods elongate usually by the axial elongation and fission of their components, and that branching results by occasional extra-axial elongation of one or more of these. The cell thus elongating sidewise rather than endwise is supposed to continue to elongate and subdivide in the direction of the new axis, forming a new chain of coherent cells at an angle with the mother chain. (See Figs. 6, 16 to 21 and 31, 32.)

Hypothesis four: That whether Hypothesis two or Hypothesis three be correct, the branching is connected in some undefined way with the large granules, sometimes polar, sometimes medial, so characteristic of *B. diphtheriæ*. The particular granules meant are, the writer believes, those which take the reddish tint when Lœffler's methylene blue alone is used as a stain, probably the same granules which with Neisser's stain stand out prominently as a deep blue or black. (See Figs. 17 to 24.) This hypothesis, referred to by the writer in his first article [18] (1898), has not been, so far as he is aware, yet placed on a definite basis. It has been suggested to the writer in a private communication that these granules may be intimately connected with the reproduction of *B. diphtheriæ* in all cases; that new rods develop always from or in connection with such granules in another rod; that such development is usually in the axis of the mother rod and so gives rise to appearances quite compatible with the accepted ideas of multiplication by ordinary fission; that occasional extra-axial development from a polar terminal granule results merely in a bent or V-shaped rod, while extra-axial development from a medial granule gives the appearance of branching. (See Fig. 18 and compare Figs. 16 to 21 with Figs. 25 to 30.)

Hypothesis five: That the branching is merely an exaggeration, accidentally symmetrical, of the swellings, projections, and general irregularities of outline well known to occur in degenerate or dying cultures of many bacterial species. (See Fig. 8.)

The physiological or developmental origin of the branching is accounted for on hypotheses connected intimately with the above hypotheses of its nature.

The first two of these hypotheses of origin hold that branching is a part of the normal active life history of the organism, contributing to its struggle for existence, but of only occasional occurrence under conditions not well understood; that it is a development in an irregular direction of forces similar to those usually resulting in simple fission; that the irregularity of the operation of these forces in this direction indicates their relative weakness and lack of organization, and that they are therefore either remnants of forces once much more constant in operation, perhaps even the most prominent ones (the reversionary hypothesis) or that they are evidences of a now developing tendency towards a more complicated existence (the evolutionary hypothesis).

The third hypothesis of origin, based on the belief that the branching is purely degenerative, traces it to the auto-intoxication by excretory products, loss of oxygen, exhaustion of food, water, etc., occurring in old cultures — in short, to the usual conditions supposed to account for involution in general.

Before giving the writer's results and conclusions, attention must be directed to the work of Nakaniski,[5] on which the third hypothesis of the nature of branching is based. He claims to have determined the presence of a nucleus or nucleus-like body in many bacterial cells, including those of *Bacillus diphtheriæ* and *Bacillus variabilis lymphæ vaccinalis*. He maintains that the smaller forms of these bacilli are single cells, the larger are chains. It seems that some of his work was done as mentioned, not on *Bacillus diphtheriæ* itself, but on the closely allied species, which he at one time[11] considered to be the bacillus of vaccinia, a claim which

was afterwards withdrawn.[11] It is probable, however, that his conclusions are as applicable to one as to the other. He describes fission as resulting, before complete separation, in the well-known "double-header" or cuneiform bacilli, which he regards as two cells. He describes and figures also the enlargement and further separation of these cells, and the gradual development of parallelism of their axes, explaining the "box of cigars" formation so often described in stained preparations. (See Figs. 31, 32.)

The writer's observations were made partly on ordinary dried and stained preparations, partly upon unstained organisms while in the process of development on the surface of an agar "hanging block." They deal only with the gross morphology. Whether the larger diphtheria bacilli are composed of one or several cells the writer cannot say from personal observation. One branching form observed seemed to carry out, so far as it went, Nakaniski's view, and the other observations neither confirm nor confute it. (See Figs. 6 and 7.)

The figures in Plate VI. represent some reproductions from sketches made to scale by the writer directly from measurements with a micrometer eye-piece upon developing diphtheria bacilli of ordinary morphology, culture reactions, and virulence to guinea-pigs.

Figs. 1 to 5 indicate the growth and division, by suddenly snapping across, of a single rod, resulting in new rods lying at an obtuse angle. The subsequent enlargement, increasing parallelism, and repeated division of the new rods is shown. The sudden snapping across of the rods and their subsequent angular positions are very characteristic features of ordinary multiplication as seen in these preparations.

Figs. 6 to 8 indicate the development of "branching." As the most satisfactory example yet seen by the writer, the conditions of development of Fig. 7 are described in detail.

The "hanging block" actually observed was made at 11 A.M., December 13, 1901, from an agar culture then five days old which had been grown in the 37° C. incubator for forty-eight hours and thereafter at room temperature. Ac-

tive multiplication under the microscope began at 11.20 A.M. and continued until the observation ceased. The branching form (Fig. 7A) was stumbled upon accidentally at 4.40 P.M. and at once drawn. Thereafter this form was constantly under observation until 8.30 P.M., when the writer was called away.

The following deductions from these observations and others on stained preparations seem to be in order:

First. — Branching of *Bacillus diphtheriæ* may occur on agar and may begin within five or six hours of inoculation under the conditions described.

Second. — It may occur when active multiplication in the ordinary way is going on all over the same preparation.

Third. — Active multiplication by apparent fission may be going on in a different part of the same rod which is also branching.

Fourth. — After the branch is formed, it may separate from the parent stem.

Fifth. — That portion of the parent stem from which the branch originates may become faint, shrunken, and passive.

Sixth. — Branching is not necessarily a matter of accidental optical apposition, for its gradual development may be watched; in Fig. 7 the branch snapped over (D, E) from the stem, the snapping occurring under the eye of the observer; obviously it must have been attached or it could not have snapped off.

The final oval form of the branch recorded was exactly similar to several oval forms seen by the writer to undergo development, and it is not unfair to assume that this body might undergo such changes. Fig. 4 represents observations on a somewhat similar body, previous developmental history unknown, made some weeks previously.

Fig. 8 indicates a form of apparent degeneration, giving rise to projections simulating branches, in the sense defined in this article.

Here it would seem that a dead or dying rod was under observation. No appreciable increase in length occurred. The separation into the two portions shown (B) occurred

gradually, without change in the relative positions of the separated portions. The bacillus (A) was at first fairly definite in outline and density. It became (B) swollen, granular, and faint, some of the granules being very fine. Only the larger granules are figured. Then it became shrunken (C), breaking up slightly, and developing two distinct projections, granuled at their extremities. The granules were refractive points, bright or dark as the focus changed. Their relation to the granules staining reddish with Loeffler's methylene blue was not determined. Active multiplication was proceeding in other parts of the same "hanging block" preparation.

These observations, few and imperfect as they are, so far confirm, explain, and add to the observations already made by various observers that the following conclusions seem justified.

First. — Passive degenerative changes in dead or dying bacilli may give rise in this species to slight irregular projections which distantly simulate branches, using this term in the wide sense already defined in this article.

Second. — As a part of the active development of the diphtheria bacillus, active branching by apparent budding, ending in the production of an oval or elliptical body, probably capable itself of further development and the production of new rods, may occur in very young cultures, the parent stem then degenerating.

Third. — As a part of the active development of the diphtheria bacillus, branching similar to that described, but terminating in an ordinary diphtheria rod-like body, and without any degeneration of the parent stem at the point of origin, may occur within twenty-four hours of inoculation; and this new rod may segment in the ordinary way, or itself produce branches, terminating in rods similar to itself or in oval bodies such as are described above.

Fourth. — Various modifications of all the processes of branching described probably exist.

Fifth. — The origin of the active multiplicative branching may be reversionary or evolutionary or merely due to

special conditions of growth not understood at present. According to the latter view, the active forces usually resulting in fission may at times undergo a lessening of tension or some other modification which results in a change in direction of their activities. Whether such a change is regressive, "a degeneration of forces" as phrased by Sedgwick, or progressive, an "ascension of forces" has not yet been determined.

[Whether the force manifested in branching proper indicates progression or regression in the individual does not affect the question as to whether the possession of such a force indicates in itself reversion or evolution in the species. Two distinct problems are involved. It may be possible to determine that problem relating to the individual; that relating to the species must probably remain speculative.]

Review of Literature. — In May, 1900, Hektoen reviewed exhaustively the literature relating to all branching bacteria without coming to any very definite conclusion, and since that time the writer has been able to find very few articles relating to the subject. In 1900 Skschivan [2] described branching plague bacilli at some length, and Galli-Valerio [8] and Conradi [9] described branching glanders bacilli. Reichenbach [3] in 1901 described branching in spirilla, and recently Meyer,[4] after a study of the *Bacillus cohærens* and other branchers, concludes that branching is a "rudimentary and infrequent reversion to ancestral types." He finds branching in young cultures, agreeing in this with Gorham and the writer, and he feels justified on this ground in believing that the bacteria in general furnish in this particular an example of correspondence between phylogeny and ontogeny, the development of branches in the young cultures being analogous to the development of branches in the species itself at an early stage in its history. His view of the branching process itself apparently corresponds with that of the second hypothesis already given. Nakaniski, in 1901,[5] described for *Bacillus diphtheriæ* and allied forms the process referred to in Hypothesis three. He says that further investigation is required. A. Fischer and others have

maintained Hypothesis four. With the exception of Gildersleeve,[6] who, according to Bergey, maintains somewhat Hypothesis three, and the writer, no other Americans have devoted much attention to the subject, so far as the writer is aware, except Craig in 1898 and W. H. Smith in 1900, who both describe branching tubercle bacilli.

Branching other than purely involutionary has now been recorded, according to Chester,[7] for twenty organisms previously supposed to be ordinary bacilli (in the sense of unbranched cells), including those of actinomycosis, tuberculosis (human and avian), glanders, leprosy, diphtheria, etc. Plague bacilli also branch (the earliest reference which the writer has been able to find being in an article by Klebs in 1898). The writer also found branching in a diphtheria-like organism, non-virulent and growing with luxuriance on agar, isolated from the nose of a horse and perhaps identical with Nakaniski's *Bacillus variabilis lymphæ vaccinalis*.

In a preliminary note[13] the writer called attention to a possible connection between the branching of diphtheria bacilli and the metachromatic granule so often found in the parent stem at the base of the branch. But, as was then pointed out, such a granule is by no means always present. Further observation leads to doubt that the connection is very close. Granules, indistinguishable from these, are very common in many types of diphtheria cultures, and are as common in those which do not branch as in those that do. In the branched forms the granules may not be present at all, or may be present in some other situation than at the base of the branch. A granule may be present at the base of a constricted branch, sometimes at the bases of two branches springing from a single point, and no granule may exist at all at the base of a non-constricted branch. The observations of Nakaniski[5] also seem to negative this view. Finally, although the second or third hypothesis may suffice for the diphtheria bacillus, the branching of other bacilli like *Bacillus pestis* remains to be cleared up.

Nomenclature. — Following various previous writers, the

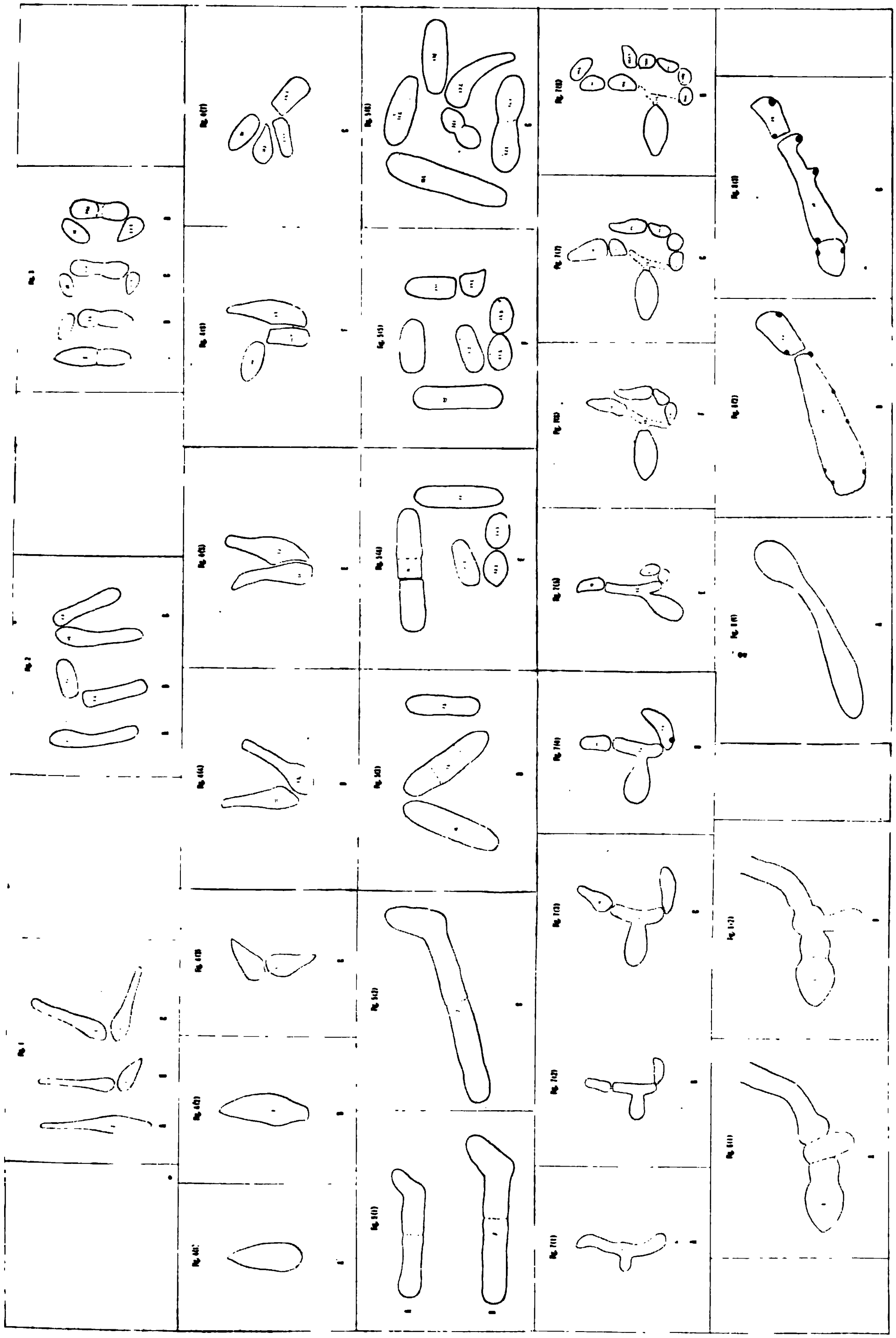
writer, in 1899, advocated the name streptothrix for the diphtheria organism, but feels now that in common with others at the time his ideas were far astray. Bacterial nomenclature should follow as far as possible the canons of the botanist. It is easy to see that the whole subject needs much development before consideration of the names to be employed is necessary. Names, indeed, cannot be intelligently selected until the facts themselves are thoroughly established and correlated. Moreover, even the advisability of placing branching bacteria in any class distinct from the forms not yet known as branching seems questionable. It is not improbable that it will ultimately prove simpler, as Meyer maintains,[4] to readjust the definition of the term bacillus, since so many of this group show branching at times, rather than to make any more radical revision. Chester,[7] in his recent work, classes diphtheria bacilli as myco-bacteria, a step which, while proper in so far as it recognizes the branching peculiarities as of importance, is perhaps a little premature.

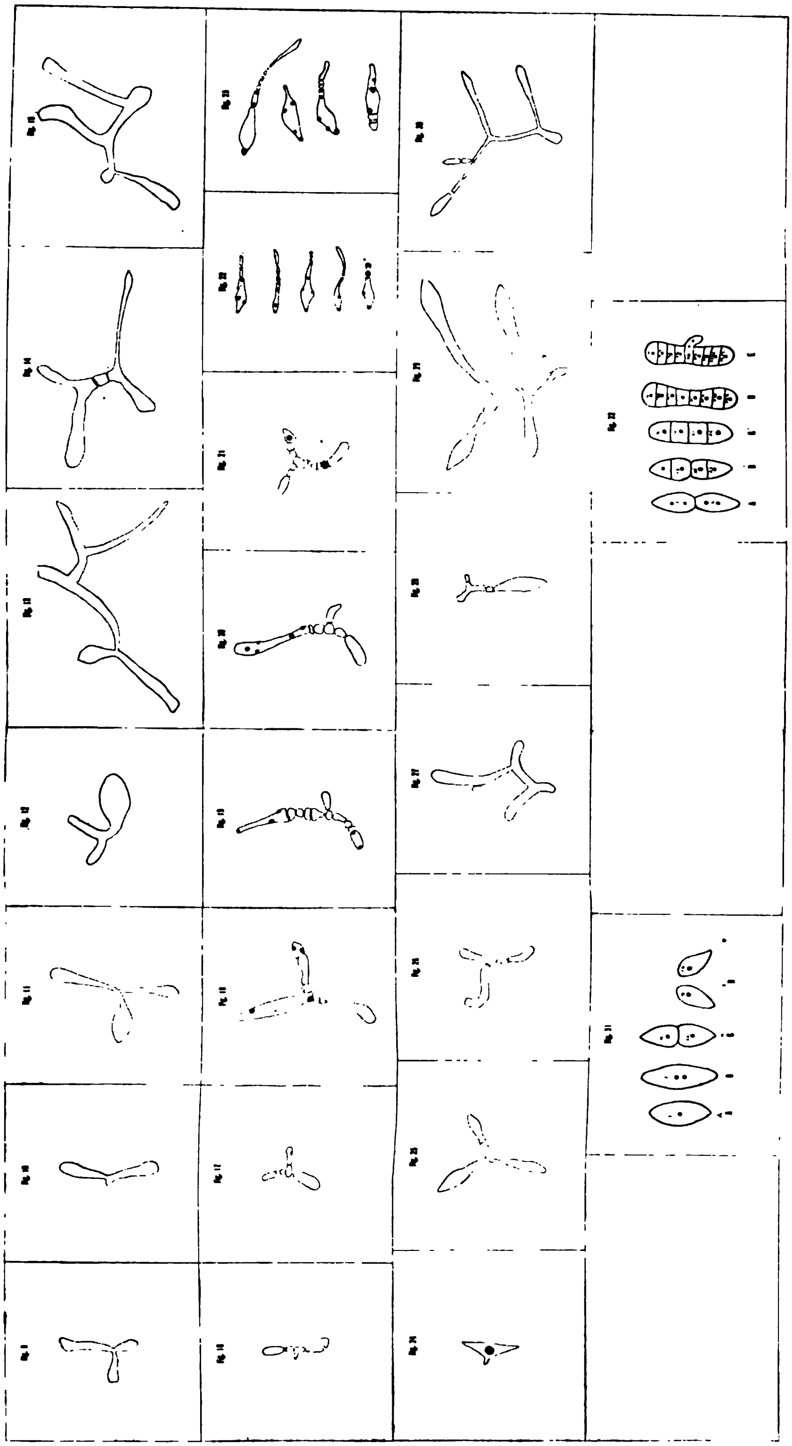
DESCRIPTION OF PLATES.

Plate VI. shows drawings to scale from micrometer measurements of individual diphtheria bacilli during the process of growth on the surface of a nutrient agar "hanging block" in a warm stage under the microscope; Zeiss, ocular five, objective one-twelfth, oil immersion; Welsbach light, concentrated by a four-inch lens of seven-inch focus. The individuals grouped under each figure were all drawn to the same scale; the scale was not the same, however, for all the different figures. Figs. 1 and 3 are comparable to each other; Figs. 2 and 4; and Figs. 5, 6, and 8. Fig. 7 is drawn to one-half the scale of Figs. 5, 6, and 8.

Plate VII. shows drawings, not to scale, of dried and stained preparations of diphtheria bacilli found in serum and agar cultures at various times. Figs. 31 and 32 are copies from Nakaniski's plates.

The bacilli observed came from cultures of *Bacillus diph-*





theriæ obtained in the routine diagnostic work of the Boston Board of Health.

Figs. 1 to 3, 27, 29, and 30 were from McKenna case (virulent bacillus in mild clinical case).

Figs. 4 to 7 were from Dias case (virulent bacillus in well person).

Fig. 8 was from Raymond case (virulent bacillus in well person).

Figs. 16 to 26, and 28 were from Charak case (virulent bacillus in mild clinical case).

The agar "hanging block" preparations in which development was actually observed under the microscope (Figs. 1 to 8) were never more than ten hours old from the time of inoculation. Some were inoculated from serum cultures, some from agar, these cultures being of various ages up to several days. The cultures from which came the dried and stained preparations represented in outline (Figs. 9 to 30) were not more than twenty-four hours old at the time of making the preparations. Figs. 9 to 15 were from serum cultures about twenty-four hours old. Figs. 16 to 26, and 28 were from agar cultures seventeen hours old. The bacilli, in all cases, were grown at 37°C. The genealogical relationships are indicated in Figs. 1 to 8 by an adaptation of Rickards' system of genealogical culture record.[12] The time occupied in the various stages of development was not noted in all cases. Sometimes a number of different individuals or groups were under observation at the same time, changes occurring in one group while attention was devoted temporarily to another, so that only approximate time relations could be recorded. The changes in Fig. 1 occurred in from 30 to 60 minutes, the change from A to B occurring suddenly under the observer's eye, as did also the change Fig. 3 from B to C; in Fig. 4, D became G in forty-five minutes; in Fig. 5 the times recorded beyond indicate only the times at which the changes were first noted after they had occurred, usually within a few minutes. In Fig. 6 development beyond B occurred, but was obscured to some degree by the overlapping of other multiplying bacilli and was

therefore not recorded. In Fig. 8 the passive disintegration of the bacillus gave no definite points in time for record, the drawings being made simply at those intervals when it became apparent that the changes had distinctly advanced. In Fig. 7 alone a fairly complete record was obtained, a new drawing being made whenever the smallest change could be definitely detected. Only certain of these drawings have been reproduced. The time relations for Fig. 5 were: A to B = sixty minutes; B to C = thirty minutes; C to D = fifty-five minutes; D to E = twenty minutes; E to F = seven minutes; F to G = sixty-eight minutes. Total, four hours and fifteen minutes.

The time relations for Fig. 7 were: A to B = thirty-seven minutes; B to C = thirty-two minutes; C to D = sixteen minutes; D to E = eight minutes (this change (D to E) in the relation of the branch to the parent stem occurred suddenly under the eye of the observer, the developing branch having then been under observation one hour and thirty-three minutes); E to F = one hour two minutes; F to G = thirty-seven minutes; G to H = thirty-eight minutes (about). Total, three hours and fifty minutes. The agar "hanging block" under observation had been inoculated five hours and forty minutes when the branched form was first seen; H therefore represents the condition nine hours and thirty minutes after inoculation. Active multiplication was continuous in other parts of the same preparation, being noticed twenty minutes after first focussing on the preparation, or a total of nine hours and ten minutes.

Figs. 9 to 15 are selections from drawings by the writer representing cultures of branchers observed from 1900 to date.

Figs. 16 to 30 are selections from drawings illustrating some of the relations of "granular," "barred," and "solid" types of *Bacillus diphtheriæ* to red granules and to branching. Figs. 16 to 21 seem to support Nakaniski's theory of branching, already described. Rough copies of some of his diagrams illustrating this theory are given in Figs. 31 and 32. Fig. 18 shows a red granule at the base of a branch.

Figs. 22 to 24 show the presence of red granules without branching. Figs. 16 and 25 to 30 show branching without red granules. Figs. 16 to 26, and 28 were all from the same agar cultures inoculated from the same original culture and grown under the same conditions at the same time, both granuled and non-granuled branchers being found in the same tube.

BIBLIOGRAPHY.

Hektoen's article (New York Medical Journal, May, 1900) reviews the literature of branching bacteria to that date. The following references partially cover the subsequent period:

1. W. H. Smith. Boston Society Medical Sciences, 1900.
2. Skschivan. Central. f. Bakt., XXVIII., No. 10–11, September, 1900.
3. Reichenbach. Central. f. Bakt., XXIX., No. 13, April, 1901.
4. Meyer. Central. f. Bakt., XXX., No 2, July, 1901.
5. Nakaniski. Central. f. Bakt., XXX., No. 3, July, 1901.
6. Gildersleeve. Quoted by Bergey, "Medicine," Detroit, August, 1901.
7. Chester. Manual of Determinative Bacteriology, 1901.
8. Galli-Valerio. Central. f. Bakt., XXVIII., No. 12–13, October, 1900.
9. Conradi. Zeitsch. f. Hyg., XXXIII., Heft. 2, 1900.

OTHER ARTICLES REFERRED TO IN THE TEXT.

10. Wesbrook. Transactions American Public Health Association, 1900; Transactions American Association Physicians, 1900.
11. Nakaniski. Central f. Bakt., Bd. XXVII., No. 18–19; Central. f. Bakt., Bd. XXVIII, No. 10–11.
12. Rickards. System of Recording Cultures Genealogically. Journal Medical Research, 1902.
13. Hill. Journal Boston Medical Sciences, January, 1899; Transactions American Public Health Association, 1900; "Hanging Block" Preparations for Microscopic Observation of Developing Bacteria. Journal Medical Research, 1902.

OBSERVATIONS UPON THE MORPHOLOGIC VARIATIONS OF CERTAIN PATHOGENIC BACTERIA.

A. P. OHLMACHER, M.D.,
Professor of Pathology, Northwestern University Medical School, Chicago, Ill.
(*From the Department of Pathology, Northwestern University Medical School.*)

It is the purpose of this communication to present in as condensed a form as possible the following observations:

I. Upon some experimentally-induced morphological variations in *B. diphtheriæ.*

II. Upon an example of unusual cultural variation in *Strept. pyogenes.*

III. Upon an example of extreme polymorphism in *B. coli.*

I.

The renewal of the discussion upon the morphological variations of the diphtheria bacillus and its bearings upon the identity of the so-called pseudo-diphtheria bacillus prompts me to now record some experiments along this direction made in Cleveland during the winter of 1894. Of the series of experiments then prosecuted having for their object the study both of morphological and physiological features, only those bearing upon the morphology will be discussed briefly here, although the upshot of the complete investigation was such as to emphasize the fact that the organism of diphtheria is subject to a wide range of variability in which most of its common characteristics are unstable; and even at this time I reached the conclusion that the microbe designated pseudo-diphtheria bacillus was but a modified variety of the Löffler bacillus.

Three distinct cultures were used, obtained by the routine diagnostic method from the throats of individuals suspected of having diphtheria. For convenience these cultures will be designated as Bacillus A, Bacillus B, and Bacillus C. The

culture medium, both bouillon, agar, and Löffler's, was all of one lot kept under uniform conditions. Control tests to determine the influence of such factors as moisture of the media, temperature of the incubator, difference in isolated colonies, and so forth, were made, so that I think these possible sources of error may safely be eliminated.

Bacillus A was recovered from the throat of a child at the onset of what proved to be a fatal case of diphtheria. B and C were obtained from two children suffering with pseudomembranous angina, both patients recovering in a few days without specific treatment.

Bacillus A from the original source, grown for repeated generations on Löffler's serum, maintained the form of a long, granular, or barred bacillus (Types C and C_1, of Westbrook[1]), with frequent spherical or fusiform (clubbed) ends. It measured four and one-half to seven and one-half mikrons in length, and one and one-half to two mikrons in thickness. In bouillon it became a short, granular bacillus, and on agar shorter still, but resumed its usual form on return to Löffler's medium. (Its form after several generations on Löffler's serum is shown in Fig. 1, Plate VIII., drawn from a careful camera lucida tracing with Leitz obj. $\frac{1}{12}$ oil immersion, oc. 4.) The original culture was actively pathogenic for guinea-pigs, killing half-grown animals in thirty-six to forty-eight hours in a dose of one-half a cubic centimeter of a twenty-four-hour bouillon culture. On recovery from the organs[2] of guinea-pigs the bacillus resumed the shape shown in the original cultures when grown on the serum mixture.

The particular transformation in morphology which I desire to emphasize was obtained by design in the following manner: From one of the earlier and still pathogenic cult-

---

[1] Trans. Assoc. Am. Phys., 1900.

[2] At this period (December, 1894) the author made use of a procedure original with him and now well known for obtaining the diphtheria bacillus from the viscera of inoculated guinea-pigs, consisting in sowing relatively large pieces of the organs (liver, spleen, adrenals, and occasionally kidneys) in bouillon, incubating twenty-four to forty-eight hours, and then, after breaking up the tissue fragments, inoculating Löffler medium from the suspension.

ures a twenty-four-hour bouillon culture was prepared, and of this one-half a cubic centimeter was injected beneath the shaved and disinfected skin of a white rat, the puncture being sealed with a collodion dressing. The bacilli in serum cultures from the bouillon used for inoculating the rat still took on the long granular type. In forty-eight hours the injection site presented an area of induration two centimeters in diameter, and after carefully disinfecting its surface it was incised, and several tubes of Löffler medium were inoculated from the tissue juices. Pure cultures of the bacillus were secured which had the usual physiological attributes of *B. diphtheriæ*, except that acid[1] was produced in glucose-litmus-agar. But in the morphology of its subsequent serum cultures, Bacillus A, as recovered from the rat's tissues, took on an entirely different type. It had now become a short, plump, uniformly staining rodlet, an example of the short, solid type of diphtheria bacillus of Westbrook, and of Gorham,[2] which, by many authorities, is classed as the pseudo-diphtheria bacillus. It now measured about two mikrons in length, and one mikron in thickness. Only occasionally could a short barred rod be found. (Its shape is seen in Fig. 2, Plate VIII., and may be compared with Fig. 1, since it was drawn to the same scale.) This experiment was twice repeated with identical results, and from it I conclude that in this particular instance a long, granular, so-called "typical" diphtheria bacillus was converted into a short, solid, atypical (or pseudo-diphtheria) bacillus by a brief existence in the tissues of an immune animal: thus apparently confirming Gorham's[3] suggestion that the change from the granular to the solid type, as encountered clinically, is produced by sojourn in an immune or partially immune human host.

Bacillus B was, at the outset, a short, plump, solid rodlet with an occasional short granular example; its average

[1] The production of acids and alkalis in glucose, lactose, and saccharose agar was found to be quite variable during the course of the experiments with these three types of diphtheria bacillus.

[2] Jour. Med. Research, Vol. VI., No. 1, 1901.

[3] Loc. cit.

length was two and one-half mikrons, thickness three-quarters to one mikron. Contrary to the general rule for the short, solid types, it proved highly virulent, killing guinea-pigs in twenty to thirty hours in one-half cubic centimeter doses of a twenty-four-hour bouillon culture. Both it and Bacillus A were used in the preparation of diphtheria toxins produced under like conditions, and that from B was considerably the more potent. On Löffler serum it maintained the shape shown in the original cultures, and its morphology on serum was only altered after its passage through a guinea-pig and recovery from the spleen and liver, when it became a long, barred, or granular, "typical" diphtheria bacillus, three and one-half to five mikrons in length, with swellings and clubbed ends. (In Fig. 3, Plate VIII., the original short form of Bacillus B is shown, and in Fig. 4, after its recovery from a guinea-pig's liver.) It had here been possible, therefore, to alter a short, solid, atypical diphtheria bacillus to make a long, granular form, by a single passage through the organs of a susceptible animal.

Bacillus C represented the extreme atypical morphological type in its serum cultures from the original source. It was a very short, oval or lanceolate, plump bacillus, staining solidly, and showing a distinct tendency to arrangement in parallel rows. The average length was two mikrons; thickness, three-fourths mikron. It was, in fact, a characteristic specimen of what most authors describe as pseudo-diphtheria bacillus. As obtained from the original source its pathogenic effect was considerably less than that of Bacillus A or B, seven days being required for it to kill a half-grown guinea-pig with the usual dose, and here it produced the local induration and necrosis at the injection site characteristic of atypical or attenuated diphtheria bacilli. Recovered from the spleen and liver of the guinea-pig, however, Bacillus C was no longer a short, solid rodlet, but a long, barred, or granular "atypical" diphtheria bacillus, measuring three to five mikrons in length. (Fig. 5, Plate VIII., represents it as seen in the original Löffler cultures, while Fig. 6 shows its

modification after recovery from the guinea-pig's organs.) It probably was more than a coincidence that simultaneously with the striking alteration in morphology effected by its passage through the susceptible animal, the virulence of the culture was accentuated to a point at which it killed guinea-pigs in twenty-eight to thirty hours in the same dose as employed to kill the first pig in seven days. Here again we seem to have a confirmation of Gorham's conclusion that the bacillus of diphtheria takes on the long granular form in the body of a susceptible host.

II.

The streptococcus concerned in the second observation was obtained in Cleveland, in December, 1895, by the diphtheria culture-test, from a case of follicular tonsillitis in an adult. The organism was isolated in pure culture, and, from its physiological reactions, classified as *Streptococcus pyogenes*. It was pathogenic for mice. In the original culture on Löffler's medium it took so peculiar a shape as to be confusing, and to lead to a doubtful diagnosis concerning the diphtheritic or non-diphtheritic nature of the case. In seven subsequent generations, during which the streptococcus, in pure culture, was grown on ordinary Löffler's serum, it took on the peculiar shape noted in the original culture. The Löffler mixture was in no way peculiar, samples of two distinct lots being used, and the cultures were kept both at the room and incubator temperature with no effect upon the peculiar morphological condition shown by this streptococcus. In other words, it was subjected to the ordinary laboratory conditions which, in themselves, should not be held responsible. In bouillon this streptococcus produced a diffuse cloudiness, and here it regained the usual streptococcus form, making long chains composed of small diplococcoid members like that ordinarily seen in the variety called *Streptococcus longus*. This streptococcus form was restored in bouillon after each of the seven transplantations from Löffler serum.

The remarkable feature about these experiments was the polymorphism of the organism in Löffler cultures, for in each generation the streptococcus assumed a bacillus-like form, resulting in the production of short and long straight rods, rods with swollen centers, rods with pyriform ends, with spherical ends, and with clubbed ends, all much longer and thicker than the normal elements of a streptococcus chain. Mingled with these bacillary forms were oval and coccoid elements all much coarser than those of the bouillon cultures. In preparations made with reasonable care the linear continuity of these polymorphous organisms was retained, assisting somewhat in identifying them, but the chains were readily broken and the scattered individual elements were then indistinguishable from bacilli, and the irregularities in shape most strongly recalled the diphtheria bacillus. (In Plate IX. this organism is depicted; Fig. 1 is a photograph with Zeiss Achromatic obj. $\frac{1}{12}$ in., comp. oc. 2, of the third generation of this streptococcus in bouillon; while Fig. 2 is a photograph under like conditions of the Löffler culture prepared from this bouillon. In Fig. 3 a tracing with the camera lucida was made of a preparation from a bouillon culture amplified by Leitz obj. $\frac{1}{12}$ in., oc. 4; and Fig. 4 shows some of the bacillary forms of a Löffler culture drawn under the same conditions as Fig. 3.)

That pyogenic and erysipelatous streptococci are subject to morphological variation has frequently been noted, and even that in which a bacillus-like form is assumed has been described by Rodet.[1] A point of considerable practical importance is also involved here, as must be evident when we consider the readiness with which these bacillary streptococci might be mistaken for the diphtheria bacillus when assuming the irregular forms with clubbed and swollen ends, and particularly when encountered during the prosecution of the diphtheria culture test, as was the case with me. The danger of this error was appreciated by Babes,[2] who met with these

[1] De la variabilité dans les microbes, Paris, 1894, p. 28.
[2] Zeitschr. f. Hygiene, Bd. 26, 1895, p. 418.

polymorphous streptococci, and who warns one to be on guard against mistaking them in diphtheria diagnosis.

III.

The colon bacillus making the subject of the third observation was obtained from an early autopsy in a case of gangrenous cholecystitis and cholangitis following the impaction of a large biliary calculus in the common bile duct five days before death. This case was met during my service at the Ohio Hospital for Epileptics in January, 1900, and the biliary and hepatic local infection was complicated by a colon bacillus septicemia, so that the colon organism was secured in pure aërobic culture not only from the gall-bladder, gall-ducts, liver substance, and intra-hepatic ducts, the inflamed peritoneum about the gall-bladder, but also from the heart's blood, spleen, and kidneys; while from the lungs, the staphylococcus aureus, and from the retroperitoneal glands the aureus and colon bacillus were mixed. In the first generations of the bacillus on agar plates it showed the peculiar morphological variations to be described, this being most pronounced in cultures from the hepatic apparatus, but also showing in a striking manner in the growths from the heart's blood. It required some three or four transplantations through bouillon to "rejuvenate" or restore this bacillus to the usual morphological condition of B. coli. In its physiological reactions as tested by the routine procedures, it gave the characteristics of the typical colon group, even while still irregular in morphology. It was highly pathogenic for guinea-pigs. (I lay some stress upon the fact that this polymorphous bacillus appeared in aërobic plate cultures from the several anatomical sources mentioned, to allay the suspicion that it might have been mistaken for one of the filiform anaërobes recently described in connection with biliary infections. That the morphological variation was not confined to single colonies was also established, and the possibility of the culture media being responsible was excluded by control tests with other stock colon races.)

In the original smears from the gall-bladder and bile-ducts, and the first generation of cultures from these sources, the polymorphism of this bacillus reached its extreme, though, as has been said, it was prominent in cultures from more remote regions. The task of making a detailed description of all the variations assumed by this bacillus is well-nigh impossible. But in a general way it may be said that all gradations from minute coccoid or diplococcoid to long, coarse, filamentous forms were observed. The coccus, diplococcus, and short streptococcus-like individuals corresponded precisely to those recently described by Adami, Abbott and Nicholson,[1] being often so small as to tax the amplification of the $\frac{1}{12}$ inch objective. On the other hand, some of the thick, filiform individuals, even after the breaking suffered in smearing, could be traced through three or four fields of the $\frac{1}{12}$ objective. Other threads were thin and delicate, some being quite shadowy; some of the shorter ones showed a row of deeply-staining inclusions, and others still had clear, unstainable spaces, or areas with metachromatic staining. These filaments were often aggregated into groups, several times appearing as a mass of tangled threads. Some of the shorter rods in the early heart's blood cultures *showed lateral buds, and short, true branches.* Some of the filaments or longer rods had median swellings, others among the shorter rods had clubbed ends, and many were bent at various angles.

While quite familiar with the ordinary range of morphological variation shown by colon bacilli, I was forcibly impressed by the truly astonishing character of the organism in this case, and a few years ago would have concluded that some unaccountable error in technic was responsible, and that not a single bacterial species, but a mixture of a streptococcus, a bacterium, and a filamentous organism, was here concerned. I am, however, quite positive as to the reliability of my technic, and the recent literature affords confirmation for the conclusion that in this case we are dealing simply

[1] Jour. Exp. Med., Vol. 4, Nos. 3 and 4, 1899.

with an example of extreme morphological variation in a bacillus of the colon group. In my opinion the polymorphism of this bacillus must be ascribed to certain environmental influences resulting from its abode in the biliary apparatus of the human host, and the atypical forms shown by the organism from other sources like the viscera and heart's blood would indicate that these structures were infected from the primary foci in the gall-bladder and liver, by bacilli already modified in morphology. It has been noted that the shape of the colon bacillus is altered in the diseased gall-bladder, particularly by Rodet;[1] and Adami and his co-workers[2] have shown that bile has a modifying influence in determining the morphology of the colon bacillus. Dunbar[3] has described chain formation in B. coli, and Rodel, Livingwood,[4] and Adami have produced interesting morphological variations in colon bacilli by various experimental procedures like elevated temperature, special media, the use of organic juices, and by animal inoculations.

[1] Archiv. de phys. norm. et path., 1896, p. 968.
[2] Op. cit.
[3] Zeitschr. f. Hygiene, 1892, p. 485.
[4] Cent. f. Bakter., 1898, p 980.

FIG. 1.

FIG. 2.

FIG. 3.

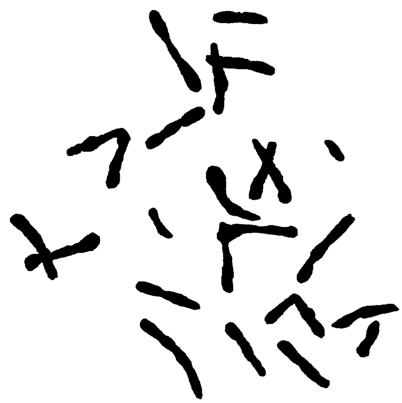


FIG. 4.

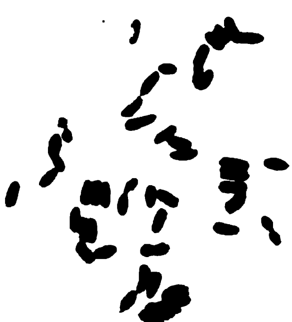


FIG. 5.

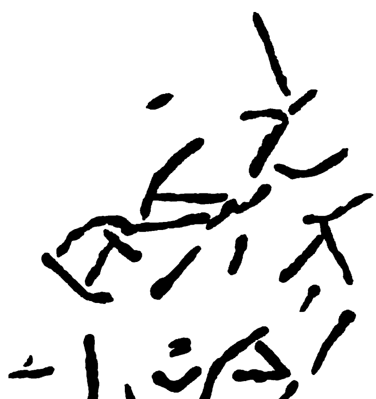


FIG. 6.

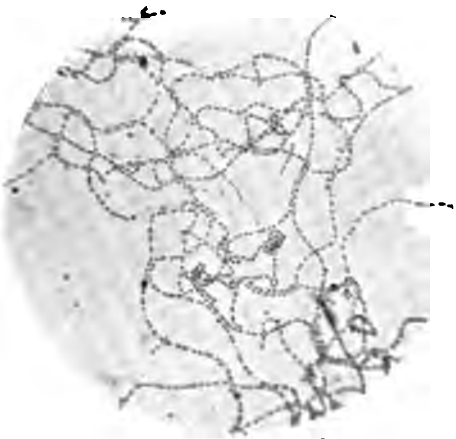


FIG. 1.

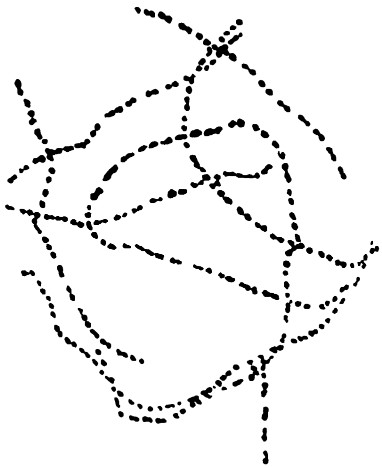


FIG. 3

FIG. 4

FIG. 2

EXPLANATION OF PLATES

ACCOMPANYING DR. OHLMACHER'S ARTICLE ON MORPHOLOGICAL VARIATIONS OF CERTAIN PATHOGENIC BACTERIA.

PLATE VIII.

FIG. 1. A long, granular, diphtheria bacillus; sub-cultures on Löffler's medium from original race secured from throat culture. (Bacillus A of text.) Camera lucida tracing with Leitz obj. $\frac{1}{12}$ in. oil immersion, ocular 4.

FIG. 2. Same bacillus growing on Löffler's medium, after recovery from the subcutaneous tissue of a white rat. It is now a short, solid form.

FIG. 3. A short, solid, diphtheria bacillus as grown on Löffler serum from throat culture. (Bacillus B of text.)

FIG. 4. Same race as Fig. 3 after recovery from organs of a guinea-pig, now assuming the long, granular type.

FIG. 5. A very short, solid diphtheria ("pseudo-diphtheria") bacillus on Löffler's medium from throat culture. (Bacillus C of text.)

FIG. 6. Same race as Fig. 5, from Löffler's serum, after passage through guinea-pig and recovery from organs. It has now became a long, barred, and granular "typical" diphtheria bacillus.

PLATE IX.

FIG. 1. Photograph with Zeiss achromatic obj. $\frac{1}{12}$ in., comp. oc. 2, of the streptococcus described in text as it appeared in bouillon cultures.

FIG. 2. Photograph as in Fig. 1, under like conditions, of the streptococcus as it appeared in cultures on Löffler's medium.

FIG. 3. Camera lucida tracing with Leitz obj. $\frac{1}{12}$ in. oil immersion, oc. 4, of the streptococcus from bouillon.

FIG. 4. Same as Fig. 3, showing some of the polymorphous bacillary forms assumed by this streptococcus when growing on Löffler's medium. Drawn to scale with preceding figure.

A RAPID METHOD FOR THE DIFFERENTIAL STAINING OF BLOOD FILMS AND MALARIAL PARASITES.

JAMES H. WRIGHT, M.D.
(*Director of the Clinico-Pathological Laboratory of the Massachusetts General Hospital.*)

Since Romanowsky, in 1891, described a method of staining the malarial parasites by which the chromatin and cytoplasm were differentially stained, various workers have brought forward modifications of his method, designed to overcome its uncertainties and to simplify it.

All of these methods, with the exception of that of Leishman (British Medical Journal, Sept. 21, 1901), have been so complicated, or so time-consuming, or so inconstant in results, or have offered so many difficulties and uncertainties in the preparation of the staining fluid, that they cannot be regarded as practical methods to be used by any but the more skillful workers.

The following method is a simplification of Leishman's method, which latter is a modification of the well-known methylic alcohol method of Jenner for staining blood films. As in Jenner's method, the troublesome fixation of the blood by heat, which is the greatest difficulty in the way of obtaining good preparations of blood by the common methods of staining, is done away with. The method gives not only a differential staining for the chromatin and the cytoplasm of malarial parasites, but it is also a general staining method for blood and as such is superior to Jenner's method, in that it sharply differentiates the nuclei and the cytoplasm of the lymphocytes and mononuclear leucocytes, as well as the granulations of the leucocytes in general.

It is confidently believed that by following out with reasonable accuracy the procedures detailed below, this simple method will not fail to give constantly the results promised for it, even in the hands of beginners in hematology.

The method is as follows:

Preparation of the Staining Fluid.

Make a one-half per cent solution of sodium bicarbonate in water in an Ehrlenmeyer flask and add to it one per cent of methylene blue (Grübler). I have found that any of the methylene blues of Grübler known as "BX," "Koch's," or "Erlich's Rectified" may be used. It seems to be important that the bicarbonate of soda be all dissolved before adding the methylene blue.

The mixture is next to be steamed in an Arnold steam sterilizer for one hour, counting the time after "steam is up." This steaming of the alkaline solution of methylene blue effects certain changes in the methylene blue whereby a poly-chromatic property is given to it, so that the compound with eosin, which is later to be formed with it, has the property not only of differentially staining the chromatin of the malarial parasite, but also of differentiating and bringing out more sharply the nuclei and granules of the white blood corpuscles. That the methylene blue must be modified in order to produce the Romanowsky stain in the malarial parasite has been generally known and the modification has been brought about by different writers in various ways, all of which either require too much time or are too difficult of execution to be satisfactory to ordinary laboratory workers.

When the steaming is completed, the mixture is removed from the sterilizer and allowed to cool, the flask being placed in cold water if desired. When it is cold, without filtering, pour it into a large dish or flask and add to it, stirring or shaking meanwhile a sufficient quantity of a one to one thousand solution of eosin (Grübler, yellowish, soluble in water) until the mixture, losing its blue color, becomes purple in color, and a scum with yellowish metallic luster forms on the surface, while on close inspection a finely-granular black precipitate appears in suspension. This will require about five hundred cubic centimeters of the eosin solution for one hundred cubic centimeters of the alkaline methylene blue solution. These are quantities which are convenient and suitable to employ.

The precipitate is collected on a filter and without washing is allowed to dry thereon. When thoroughly dry a saturated solution in pure methylic alcohol is made. Three-tenths of a gramme of the dry precipitate will thoroughly saturate one hundred cubic centimeters of the methylic alcohol in a few minutes.

The saturated alcoholic solution of the precipitate is next filtered and to the filtrate is then added twenty-five per cent of methylic alcohol; *e.g.*, to eighty cubic centimeters of the saturated alcoholic solution twenty cubic centimeters of methylic alcohol is added.

This somewhat diluted alcoholic solution of the precipitate is the staining fluid. It is permanent and may be kept on hand ready for use. Care should be taken to prevent the alcohol from evaporating and thus making the solution too saturated. The object of diluting the saturated solution is to prevent precipitations on the blood film in the process of staining.

The Staining of the Blood Film.

The films of blood, which should be spread thinly, are allowed to dry in the air. When dry, as much of the staining fluid is poured upon a film as the cover glass or slide will readily hold without draining off. Allow the staining fluid to remain in contact with the film for one minute. This chiefly serves the purpose of fixing the blood corpuscles. If the film is spread on a cover glass, the cover glass is most conveniently manipulated by means of cover-glass forceps.

Next add to the staining fluid on the cover glass or slide sufficient water, drop by drop, until it becomes semi-translucent and a reddish tint becomes visible at its margins, while a metallic scum forms on the surface. The amount of water required will vary with the amount of staining fluid on the preparation, but in general it may be said that eight or ten drops will be required if a seven-eighth inch square cover glass is used.

The staining fluid, thus diluted, is allowed to remain on the preparation for two or three minutes, during which time

the real staining of the preparation takes place, and is then washed in water.

The blood film will now be seen to have a blue or purple color, and if examined with the microscope the red blood corpuscles will be seen to be stained blue.

The next step is to develop the differential staining of the various elements in the preparation. This is done by washing the preparation in water, preferably distilled water, until the better spread portions of the film appear yellowish or reddish in color. If desired, the process of differentiation may be readily observed by placing the cover glass film-side uppermost on a slide, covering it with water, and examining it with the microscope under a low magnifying power. The red blood corpuscles, which as before stated at first have a blue color, will become greenish, then yellowish, and finally orange or pinkish in color, depending upon the depth of the original staining, which varies with the length of time that the diluted staining fluid has been allowed to act, and with the degree of its dilution.

This differentiation by washing in water seems to be essentially a process of decolorization by which some of the blue constituent of the dye is removed, for the water that drains off from the preparation has a blue color. This differentiation or decolorization proceeds slowly, and may require one to three minutes, depending upon the intensity of the staining, and upon the tint sought to be obtained in the red corpuscles.

It is apparent from the above that with a little experience with the method the color of the red corpuscles may be made either orange or pink, as the operator desires. When the desired color is obtained in the red corpuscles the preparation is then quickly dried between layers of filter paper and mounted in balsam. It is important to stop the decolorization by drying the preparation as soon as the desired tint in the red corpuscles is obtained, for it may be carried too far.

In the light of the foregoing explanations, the following summary of the method of staining blood films will be intelligible:

1. Make films of the blood, spread thinly, and allow them to dry in the air.

2. Cover the preparation with the alcoholic solution of the dye for one minute.

3. Add to the alcoholic solution of the dye on the preparation sufficient water, drop by drop, until the mixture becomes semi-translucent, and a yellowish metallic scum forms on the surface. Allow this mixture to remain on the preparation for two or three minutes.

4. Wash in water, preferably in distilled water, until the film has a yellowish or pinkish tint in its thinner or better spread portions.

5. Dry between filter paper and mount in balsam.

Dried blood films may be kept for some weeks without impairment of their staining properties. Films months old will probably not give good results.

Microscopical Appearances in Blood Films stained by this Method.

The *red cells* are orange or pink in color. Polychromatophilia and punctate basophilia (the granular degeneration of Grawitz) are well brought out. The nucleated red cells have deep blue nuclei and the cytoplasm is usually of a bluish tint.

The *lymphocytes* have dark purplish-blue nuclei and robin's egg blue cytoplasm in which a few dark blue or purplish granules are sometimes present.

The *polynuclear neutrophilic leucocytes* have a dark blue or dark lilac colored nucleus, and the granules are usually of a reddish lilac color.

The *eosinophilic leucocytes* have blue or dark lilac colored nuclei. The granules have the color of eosin, while the cytoplasm in which they are imbedded has a blue color.

The *large mononuclear leucocytes* appear in at least two forms. Each form has a blue or dark lilac colored nucleus. The cytoplasm of one form is pale blue and of the other form is blue with dark lilac or deep purple colored granules, which are usually not so numerous as are the granules in the polynuclear neutrophilic leucocytes.

The *Mast cells* appear as cells of about the size of polynuclear leucocytes with purplish or dark blue stained, irregular-shaped nuclei, and cytoplasm, sometimes bluish, in which numerous coarse spherical granules of variable size are imbedded. These granules are of a dark blue or of a dark purple color, and may appear almost black.

The *myelocytes* have dark blue or dark lilac colored nuclei and blue cytoplasm in which numerous dark lilac or reddish lilac colored granules are imbedded. In leukemia more color differences are brought out among the leucocytes than by the ordinary methods of staining.

The *blood plates* are deeply stained and are a prominent feature of nearly every blood preparation. They appear as blue or purplish rounded or oval bodies, usually of a diameter of one-third to one-half of that of a red blood corpuscle. They have ragged margins and present fine blue or purplish dots or mottlings in their substance. Sometimes they appear in groups or masses, and at first sight may be regarded as precipitates. In many instances they have the appearance of being within a red corpuscle and surrounded by an unstained zone of its cytoplasm. Occasionally a blood plate presents the appearance of being partly inside and partly outside the body of a red blood corpuscle.

While these appearances may be artefacts or due to the adhesion of blood plates to the surface of red corpuscles, their occurrence is sufficiently frequent to suggest the idea of the possibility that the blood plates represent the degenerated and extruded chromatin remains of the nuclei of the red blood corpuscles. The blood plates apparently situated inside the red cells are very likely to be mistaken by unpractised observers for young malarial parasites. This point will be referred to below.

Malarial Parasites.

The body of a malarial parasite stains blue, while the color of the chromatin varies from a lilac color through varying shades of red to almost black. In the young forms of the tertian and estivo-autumnal parasites the chromatin appears

as a spherical very dark red body, while in the older forms of the tertian parasite it has a more lilac or purplish red color and may appear in the form of a reticulum. In the intermediate forms the color of the chromatin may present variations between these extremes. In preparations examined in water mounting instead of balsam the distinct red color of the chromatin is more apparent than in the preparations mounted in balsam.

As above mentioned, the blood plates apparently situated within red blood corpuscles may be mistaken by the inexperienced for young malarial parasites. This ought never to occur if one bears in mind the fact that the young parasite of all of the three kinds should present by this method a dark red spherical nucleus and a cytoplasm which is usually in the form of a definite ring.

For those who are not familiar with the morphology of the malarial parasites, a study of the excellent descriptions and plates of the same contained in Ewing's "Clinical Pathology of the Blood" is recommended.

Various workers have shown by their modifications of the Romanowsky method that red blood corpuscles harboring malarial parasites have dark red staining granules. These granules may be brought out by the present method, but in order to bring them out it is necessary to allow the staining fluid, after the addition of the water to it, to remain on the preparation for at least five minutes and then not to decolorize or differentiate with water for as long a time or to such an extent as for ordinary blood preparations.

The thanks and acknowledgments of the writer are due to Drs. R. F. Gibson, Roger Spaulding, and F. P. Webster of the Massachusetts General Hospital for supplying him with material for experiments with this method.

ON THE BLOOD LYMPH CELLS AND INFLAMMATORY PROCESSES OF LIMULUS.

LEO LOEB, M.D., CHICAGO, ILL.

In 1879 P. Geddes[1] published some observations on the formation of plasmodia by the blood cells of evertebrates, especially echinoderms. Soon after the blood left the body the blood cells sent out processes and then united, forming one mass. He believed that masses of these plasmodia formed the clot of evertebrate blood. Fredericq[2] also believed that the first formed clot consisted of cells only. E. A. Schäfer,[3] however, showed that besides the cells, fibers were present in the clot which formed outside of the cells, and the cells were only concerned in the clot formation through the fibrin ferment which emanated from their body. A similar view was held by Halliburton.[4]

Loewit[5] investigated the blood cells of Astacus. He distinguished two kinds, one with coarse, the other with fine granules. In the cells with fine granules he described a process which he termed plasmoschisis; it consisted in part of the protoplasm with granules leaving the otherwise intact cell. He believes that mainly in this way substances originally forming a part of the cells become mixed with the blood plasma.

Hardy[6] investigated the blood corpuscles of Astacus and Daphnia. In Astacus he found three kinds of cells in the blood. First, those with basophile granulations; second, cells with small granules which disintegrated rapidly on leaving the body; part of the cell increased rapidly in size and then burst. Hardy termed these explosive cells. Third, corpuscles with coarse eosinophile granules, which showed very pronounced ameboid movement, which died in the shed blood, the pseudoporia being fixed by coagulation. The explosive cells were phagocytic, the eosinophile cells were non-phagocytic. The latter had a hyaline ectosarc and a granular endosarc. In Daphnia he found only one kind of

cell, with basophile granulations, ameboid and phagocytic. They were occasionally found to explode like the explosive cells in astacus. These facts regarding Daphnia were in the main previously observed by Metchnikoff.[7] But the process, compared by this author with an explosion, is not identical with the process described by Hardy. The observation of Geddes on the coalescence of blood cells in evertebrates, notwithstanding the possible presence of extracellular fibers in the blood coagulum, suggested a certain similarity of the evertebrate blood clot and the thrombus of mammalians, since it was shown, especially by Eberth and Schimmelbusch, that in many cases the thrombus starts as a "conglutination" of blood plates. The analogy of the evertebrate blood clot and the mammalian thrombus is still increased through the recent observations of Deetzen[8] and Deckhuizen,[9] who found that the blood plate is also an ameboid cell with nuclear substances, which, however, only under special conditions continues the ameboid movements outside the body. The analogy of mammalian blood plates and the blood corpuscles of evertebrates was emphasized by Deckhuizen.

These analogies suggested the continuation of former investigations on the blood corpuscles of evertebrates, with the addition of experiments on the behavior of these blood cells under abnormal local conditions inside the body. Such inflammatory reactions in evertebrates have hitherto been mainly investigated by Metchnikoff.[10]

Among crustacea he studied especially the behavior of Daphnia towards infections with fungi. Frequently the parasites were taken up and digested by the animal phagocytes. But, on the whole, he is impressed by the weakness of the protection by phagocytes in crustacea. Metchnikoff also found blood cells migrating to a place where the tissue had been injured, forming a capsule, and often giant cells around foreign bodies. Lubarsch[11] had negative results in regard to phagocytosis of bacteria among arthropods; he found, however, that the leucocytes collect around foreign bodies.

Adami[12] emphasizes, further, the role fixed cells play in the inflammation of evertebrates.

The following observations on Limulus were made in the summer of 1901, in Wood's Hole.* Of the blood cells of Limulus, Ray Lankester[18] gives the following description: "In measurement they closely agree, being of unusual large size for arthropods. Usually, when shed, they exhibit an oval shape, and the more elongated examples are as much as $\frac{1}{1000}$ inch long. In Limulus the corpuscles are sometimes seen with irregular ameboid processes, and when observed in the living state in the gill laminæ they very generally have an irregular outline, and may be seen to undergo change of form." Lancaster notes a number of coarse granules. The accompanying drawings show ameboid cells, with a hyaline ectosarc and a granular endosarc.

In Limulus we find one kind of blood cell, as can be seen if a gill plate is cut out and examined under the microscope. They are spindle-shaped and granular. It is of interest to note that the granules are situated in the periphery of the cell. This can be observed when a cell turns in the blood plasma; it is especially clear in case the cell is somewhat swollen. Perhaps some granules are also situated inside the cell, but this could not be definitely determined. The majority are certainly arranged at the periphery. The nucleus is situated in the center. A positive affinity of the cell granules to certain stains could not be found. Fixed with osmic acid or sublimate, the granules can frequently be stained by eosin and fuchsin, but occasionally also by anilin blue. Injection of methylene blue in different quantities and strength into the animal did not stain the granules. If the blood is examined a minute or two after it has left the body, it might be assumed that a number of different cells are present in the blood. A small nucleated cell, surrounded by a small film of protoplasm, which sends out pseudopodia is often found. Occasionally a few granules are seen near the nucleus. At some places may be seen larger cells with vacuoles, also with or without granules, with or without

* The close of the season did not permit the completion of all experiments which had been planned. Also investigations on the coagulation of the blood of Limulus, begun by Dr. O. Folin and myself, could not be completed.

pseudopodia. Many cells are united by what resembles large pseudopodia; other cells are long drawn out and still show a nucleus in the center, and granules in the drawn-out ends. In this way fibers containing granules may be produced, and many cells thus drawn out may form long continuous fibers. At other places we may see masses of granules somewhat irregularly arranged around large vacuoles, and again we may see larger or smaller collections of free granules. Frequently threads produced by the coagulation of blood are also noticed. If an observation of the blood is made as soon as possible after it is shed, and if collected in one per cent osmic acid or in sublimate, or three per cent. formalin, directly on the slide, it may be seen that all these appearances are produced by the breakdown of the one cell described above. The change which usually takes place first, and which is therefore the most difficult to observe, is the dissolution of the whole cell. The surface tension of the cell protoplasm disappears and the granules flow out into the surrounding blood plasma. That accounts for many of the scattered granules found in the coagulum, and for irregular groups of granules. At other times the diminution of surface tension is not as rapid. The cell increases very much in size, the granules move away from the nucleus, but inside at a time when the cell contour is still present the granules arrange themselves into rows and become connected by fibers. This is a second way, besides the one described above, in which fibers containing granules are formed. The other forms of cells mentioned before are the result of the combination of the following processes: 1. Partial or complete disappearance of the granules. 2. Changes in the surface tension of a part of the cell, which results in either the entire disappearance of some parts of the cell or the separation of certain drops from the rest of the cell or the protrusion of pseudopodia. These droplets can be perfect fluid, and flow around the rest of the cell. 3. In the following coagulation of the previously more liquid parts, which is especially pronounced with regard to the pseudopodia, which after being formed do no longer change their shape, even

under the pressure of the cell which may move about; they are, however, elastic. 4. The whole cell may become soft, and, under the influence of external tension or pressure, be drawn into long threads. The cell inside the body does not seem to adapt itself to the shape of narrow passages, as far as can be observed in the gill plates. If a gill plate is observed under the microscope, and pressure is exerted with the cover-glass upon the gill plate, it may be seen that many cells are destroyed, and masses of free granules appear; we also see cells with very little protoplasm, and with pseudopodia around the nuclei; occasionally, we may also see small fibers with granules. These changes may therefore take place inside the body.

The best way to preserve the blood cells I found to be an injection of about ten to fifteen cubic centimeters of three per cent formalin into the animal. After a few minutes the blood can be drawn, and in the majority of cases the blood cells are found unchanged, as one finds them in the gill plates. But even here a very small number of cells undergo the changes described above. Sometimes a little fibrin is found at the bottom of the vessel, in which the blood is collected, but the fibrin formation in this blood is insignificant in comparison with ordinary blood. However, on several occasions exceptions have been observed, a larger amount of fibrin being formed, or the cells showing greater changes. The blood also, when directly collected in three per cent formalin in a vessel or on the slide, shows the majority of cells well preserved, but under these conditions the albuminous precipitate produced by formalin may make the investigation more difficult. Five per cent carbolic acid used either by injection or on the slide, or in a vessel for receiving the blood, does not usually inhibit the changes in cells, and when it does its action is very slight. It does not prevent the coagulation of blood. If blood is collected in two-tenths per cent Sodic hydrat, we may observe the appearance of small hyaline droplets at the outside of the cell, which may flow around the cell, so that we thus have a hyaline ectoplasma and an endoplasma, which may still contain granules. In distilled water the effect is

similar, but usually not so pronounced as in diluted alkali. A solution of a mineral acid affects the cells quite differently. Cells collected in two-tenths per cent sulphuric acid, or in a weaker acid, lose all or almost all granules. The cells become round, the nucleus becomes very distinct and shows a few distinct granules. No pseudopodia appear, no drops are found around the cells. A precipitate of albumin is formed, in which most of the cells are imbedded. But occasionally outside the albuminous precipitate fibrin-like threads are found. Microscopic examination of these threads showed that they consisted entirely of round cells without any fibers between them. The cells were agglutinated. No threads were formed besides the ones consisting exclusively of these cells outside the albuminous precipitate.

In fifteen per cent Sodic chlorid solution, in glycerine and chloroform, the cells usually sent out pseudopodia, and included occasionally vacuoles; they were generally very small. Lithium, cobalt, magnesium solutions have not shown so far any specific effect upon the cells. Injection of a strong solution of Potassic hydrat into the animal itself (in one experiment, for instance, 1$\frac{3}{4}$ cc. caustic potash was injected into a young limulus, 8–10 cm. long) left at least a large number of the blood corpuscles intact. The pressure of the cover-glass upon the excised gill plates injures some blood corpuscles, so that only an approximate statement can be given with regard to this point. If we see the formation of pseudopodia-like processes in the blood corpuscles outside the body, we might believe that the blood corpuscles of Limulus are very actively ameboid. But observing these cells in the gill plates I have never been able to see any sign of ameboid movement. The observation was made at room temperature only. I also injected different quantities of suspensions of carmin in sea water into several limuli, and examined the blood corpuscles at different times up to sixteen hours afterwards. In a few cells I saw a small granule of carmin. But that was exceptional. The large mass of carmin was found near the place of injection, surrounded by coagulum. Sections made through the tissue near the place of injection, sixteen hours

after injection, showed carmin in the blood vessels, surrounded by coagulum. In one cell a granule of carmin could be seen. These results do not speak for any pronounced phagocytic activity. Phagocytic action presupposes amebic movement.

It seems possible to reduce the great variety of changes described above to the differences of intensity which, with two succeeding changes, take place in the cells. The first stage consists in liquefaction of the cell or of part of the cell accompanied by changes of surface tension, and the second change consists in a succeeding coagulation of the cell or part of the cell. If the liquefaction (which may itself vary in degree) rapidly affects the whole cell, the above described dissolution takes place. This change usually took place in the beginning, immediately after the blood had left the body. It may be suggested that it affected those cells which came in direct contact with the body wall or the glass; the cells in the center of the stream of blood were gradually affected, and the process of liquefaction took place only at a part of the cell, resulting in the formation of pseudopodia, and of droplets. This dissolution was aided by the addition of alkali, or of distilled water to the already alkaline blood. It was prevented in an acid medium. The following coagulation is demonstrated by the appearance of fibers inside of the cell boundaries during the dissolution of cells, and by the fact that these fibers were not dissolved soon afterwards; further by the above-recorded observation on the solid character of the pseudopodia after being formed, and finally by the fact that a number of cells inside and outside the clot remain usually unchanged. They probably coagulate as a whole. In many cells, in which the usual changes take place, these frequently do not proceed to the entire destruction of the cell, but cease at some intermediate stage.

In one respect these cell changes resemble the ones connected with ameboid movements; that is, that in both cases the protoplasm sending out processes is hyaline, although the granules of the intact blood cells of Limulus are situated

in the periphery of the cell. The processes of liquefaction which make the production of these processes possible usually include a dissolution of a part of the granules. In the first stages, however, one may occasionally see a blunt process of the cell still containing some granules, and very rarely the larger pseudopodia contain a granule at their base. These changes of the blood cells of Limulus may essentially be produced by processes similar to the ameboid movements of other cells, but they are not reversible. The extremes of liquefaction and of coagulation proceed too far.

In the case of the formation of processes and drops, we found a parallelism in the disappearance of the granules, and in the partial liquefaction of parts of the protoplasm in which the granules were situated. This parallelism, however, does not always exist. In the blood cells collected in acid all or almost all granules disappear, but the cell protoplasm does not become dissolved. The reverse takes place in those cells which rapidly dissolve as a whole immediately after the blood has been shed. In this case the granules mostly remain unchanged. The process of liquefaction of the cell protoplasm seems to take place so rapidly that there is not time enough for the granules to become affected. In the other cells usually a part of the granules disappears at the time during which other changes in the cell protoplasm are taking place. The cells that have once arrived outside remain to a large extent unchanged. I once saw one granule disappear in a fiber outside of a cell, but the majority remains unchanged in the coagulum in which they soon become imbedded. The conditions for the disappearance of the granules and of the rest of the cell protoplasm are therefore similar, but not identical. It is especially remarkable that so many of them remain unchanged outside of the cell. Perhaps they also coagulate.

We stated before that fibers can be formed by the blood cells in different ways, these fibers becoming sometimes quite independent of any connection with cells. The fact that here

fibers are formed from cells in a rapid way may perhaps become of some interest in regard to the question of the cause of the formation of connective tissue fibers from connective tissue cells. The origin of other fibers to be found in the blood coagulum, however, cannot be traced directly back to cells. This latter part of the coagulum is, in the beginning, not always present as fibers, but as a gelatinous mass. One fact, however, must be considered, namely, that when the blood leaves the body a number of cells, and probably a considerable number, instantly becomes dissolved. It may be that the substance of these dissolved cells contributes directly to the formation of the gelatinous blood clot.

If blood is collected on the slide, fibers can easily be produced by lifting the cover-glass from the slide and pressing it on again, repeating this process several times; the addition of distilled water seems to favor the production of fibers. The first result of these movements observed microscopically is that the cells present become drawn out and arranged in long rows. These rows of cells probably afterwards form at least a part of the fibers. With regard to the direction in which the fibers run on the slide, I could observe that this, too, depended upon a mechanical factor. The direction in which the blood flows on the slide is the same direction in which afterwards the fibers are found to be running. With regard to the causes of the changes in these cells, further experiments must be made. Suggestions for further work in this direction may be found in the above-mentioned experiments of Deelzen, in certain experiments of Quincke, and of Haycraft and Carlier, who succeeded in preserving crustacean blood in a fluid condition by keeping it suspended in oil.

Besides the investigation of the blood cells in the gill plates, or outside the body, a number of experiments were made in which foreign bodies, like silk thread, pieces of gauze, agar, or the white of an egg coagulated by heat, were introduced in the body of a Limulus, either through a

joint, or directly into the anterior segments. The wounds were closed by suture. At different periods, from a few hours up to eleven days after the insertion, pieces were removed, fixed in sublimate, and microscopic sections made. Occasionally, prior to removal, three per cent formalin was injected into the animal, in order to prevent the breakdown of the blood cells after the piece with the surrounding tissue was cut out. In pieces which were fixed and hardened, the characteristic appearance of the blood cells described above was not marked. In some cases only the granulations of the blood cells remained visible. Still, it was usually easy to distinguish blood cells from connective tissue cells, which generally had larger vesicular nuclei. But sometimes the distinction was not clear. Among the blood cells there appeared cells with large vesicular nuclei and a relatively small protoplasmic zone around them; nothing definite being known about the origin of the blood cells in Limulus, and about their relation to connective tissue cells no statement can be made regarding the significance of these cells appearing among the blood cells.

The result of all these investigations on the action of the cells of Limulus on foreign bodies, however, can be given without going into a detailed description of all investigated pieces, because the result was in the main identical in all cases. If we implant a piece of gauze or of agar in a mammal, for instance a guinea pig, we see a definite series of changes (which I intend to describe more fully in some other connection). After seven hours, polynuclear leucocytes can be observed wandering into the agar. Later on, we see connective tissue organizing parts of the agar. These cells form ramifications, so that their growth in the agar can be compared with small trees. Besides giant cells of different appearance are formed around various kinds of foreign bodies. The reaction in a Limulus towards a foreign body is quite different from this. In regard to the blood cells of Limulus, a behavior different from the action of the cells of a guinea pig was to be expected, from what we have seen be-

fore respecting the sensitiveness of these cells towards the influence of foreign bodies. I never found an immigration of the blood cells of Limulus into agar or coagulated egg which could be compared to the one which can be observed in a guinea pig. There was no formation of these tree-like ramifications. That applies as well to the blood cells as to the connective tissue cells of Limulus. After five days I found in one case connective tissue relatively well preserved directly in contact with coagulated egg, but no connective tissue cells were immigrating into the egg. The usual reaction of the blood cells towards all foreign bodies consisted in forming part of a coagulum around the foreign body and in its fissures, just as they reacted coming in contact with foreign substances outside the cavity of the body. Such a coagulum had formed a few hours after the operation. It could still be seen eleven days afterwards. A number of blood cells were frequently better preserved near the agar or gauze, which, of course, is to be expected, since a certain degree of circulation is maintained. In later periods the part of the coagulum nearest the foreign body usually becomes necrotic, nuclei and cells breaking down and disappearing. Only in a few cases were blood cells seen in the peripheral part of the egg itself, and especially in the somewhat softer agar, but here also the appearance was quite different from the one found in mammalia. It is more probable that in this case a few cells were passively pushed into the marginal fissures of the agar or egg.

Where a foreign body is inserted, or a wound is made in a Limulus, the neighboring tissue usually shows more or less necrotic changes; especially the muscles degenerate. On the first day marked degeneration of the muscle was present. (How far in a normal Limulus degeneration of tissues takes place cannot be stated.) This degeneration of the muscles seems to be connected with a gradual liquefaction, the outlines of the muscles becoming indistinct. Between such degenerating muscle, masses of blood cells may in many cases be found, and the muscle included in them gradually disappears. Whether this is caused merely by the pressure

of the surrounding blood clot, or whether proteolytic ferments are present in the fluid, cannot be decided. But occasionally we see well-preserved cells in close contact with disappearing muscles; in such a case a dissolving influence of these cells cannot be absolutely excluded. Only in a few cases could foreign particles be seen in a few cells. Regarding their origin whether from blood or connective tissue cells nothing definite can be stated.

Progressive changes of the surrounding connective tissue cells were never seen. No mitosis, no giant-cell formation could be observed. Of course, this holds good only for the time up to which the tissue was examined. The only progressive change noted was amitotic division of muscle cells which were seen at different periods, and which perhaps preceded the muscle degeneration. I have not yet been able to determine how far such nuclear divisions take place in the normal Limulus. The determination of amitotic division in connective tissue cells would be difficult. Near the necrotic hypodermis no regeneration could be observed. Although the regenerative activity under conditions called inflammatory is weak or wanting, Limulus has the power to regenerate lost extremities, as I accidentally found in an animal where the lost segments of an extremity were beginning to regenerate. In mammalia the cells usually react very strongly towards foreign bodies without having the power to regenerate lost extremities. Therefore these two regenerative processes are somewhat different as to the conditions by which they are produced.

SUMMARY.

1. Limulus possesses one kind of blood cell which has no ameboid movements, or, if any, only very weak ones, and no marked phagocytic properties.

2. The majority of the granules of this cell are situated in the periphery of the cell.

3. This cell on leaving the body responds to the stimulus of a foreign substance by a number of changes, all having this in common: that a period of liquefaction is followed by a period of coagulation. Differences of intensity of these two processes explain the different behavior of the cells.

4. The solution of the granules of the cell is only partially parallel to the dissolution of the rest of the cell. No solution of granules usually takes place outside of the body.

5. Weak alkali favors the process of liquefaction, weak acid preserves the cells. In weak acid, fibrin-like threads may merely consist of agglutinated cells.

6. A part of the fibers found in coagulated blood is formed directly from the cell protoplasm. Also in Limulus, mechanical factors influence the formation of fibers. Their direction is likewise determined by mechanical factors.

7. The blood cells of Limulus do not penetrate into agar or coagulated albumin transplanted into the body of a Limulus in a way comparable to the mammalian leucocytes. The majority is found as a part of a coagulum around a foreign body.

8. No distinct progressive changes (mitosis) in the fixed tissue cells of Limulus can be seen up to the eleventh day after the introduction of a foreign body. No giant cells are formed around foreign bodies. The surrounding tissue becomes, to a large extent, necrotic.

BIBLIOGRAPHY.

1. P. Geddes. On the coalescence of the ameboid cells into Plasmodia and on the so-called coagulation of invertebrate fluids. Proc. Royal Soc., Vol. XXX.

2. Fredericq. Note sur le sang de l'nomard. Bull. de l'Acad. de Belgique, 1879.

3. E. A. Schäfer. Proc. R. Soc., Vol. 34.

4. Halliburton. On the blood of Decapod Crustacea. Journal of Physiology, Vol. VI.

5. M. Loewit. Über die Beziehung der weissen Blutkörperchen zur Blutzerinnung. Ziegler's Beiträge, 1889.

6. W. B. Hardy. Crustacean blood corpuscles. Journal of Physiology, 1892.

7. Metchnikoff. Über eine Sprosspilzkrankeit der Daphnien. Virch. Arch., Bd. 96.

8. Deelzen. Untersuchungen über die Blutplättchen. Virch. Arch., Vol. 164.

9. Deckhuizen, M. C. Über die Thombbcyten (Blutplättchen). Anat. Anzeiger, B. XIX.

10. Metchnikoff. Leçons sur l'inflammation. Paris, 1896.

11. Lubarsch. Untersuchungen ueber die Ursache der angeboren und erworbenen Immunität. Berl., 1891 (cit. from Metchnikoff).

12. Adami. Inflammation in Albutt's System of Medicine. L.

13. Ray Lankester. Skeleto-trophic tissues and coxal glands of Limulus. Quarterly Journal, 1884.

14. Haycraft and Carlier. On invertebrate blood removed from the vessels and entirely surrounded by oil. Proc. R. Soc. of Edinburgh, Vol. XV.

This number is the Second Annual Report of the Cancer Committee to the Surgical Department of the Harvard Medical School. Its publication is paid for in large part by the funds of that Committee.

EDITOR.

THE

Journal of Medical Research.

(New Series, Vol. II.)

Vol. VII. MARCH, 1902. No. 2.

NOTES ON THE ABSORPTION AND INCRUSTATION OF ELASTIC FIBERS IN GIANT CELLS.

Ludvig Hektoen.

(From the Pathological Laboratory of Rush Medical College, Chicago.)

Peter Rona [1] gives a review of the literature bearing on giant cells and elastic fibers. Inclusions in giant cells of various sorts but undetermined nature are mentioned by Virchow, Billroth, Schüppel, Lang, Soudakewitch, and Krückmann. In some of the instances it concerned calcified concretions, but Soudakewitch [2] observed and pictured elastic fibers within vacuoles in giant cells in lupus and in tissue from "pascha-churda," a cutaneous granulomatous process in Turkestan. Some of the fibers appeared as if digested; others were split up into segments; curious, large vacuoles, with concentric markings, occurred around some of the fibers. He saw fibers enter giant cells, lose power to stain, emerge, and regain staining power (Herzheimer's method). It seems quite likely that some of the appearances described by Soudakewitch were produced by calcareous infiltration of some of the fibers included by giant cells. Rona actually demonstrated calcareous incrustation of elastic fibers in the giant cells of lupus, and furthermore that many such fibers were impregnated and covered with iron. By means of Unna's orcein method followed by Perls' iron reaction, various pictures showing this condition were obtained and some of these are illustrated in his article. Apparently the fibers acquire a special affinity for iron. Rona found ferriferous fibers in seven of ten cases of cutaneous tuberculosis, most numerously in tuberculosis

[1] Ziegler's Beiträge, 1900, xxvii, 349–358.
[2] Virchow's Archiv., 1889, cxv, 264–281.

of dependent parts where congestion and hemorrhage are favored. He found such fibers also in leprosy. Calcareous incrustation and siderosis may occur upon the same fiber. These changes were not observed outside of giant cells, hence it lay near at hand to connect them with intracellular degenerative processes. Rona failed to find such changes in atheromatous aortas and about pulmonary cavities. The giant cells appeared to be of the tuberculous type, a fact that pointed against the theory that the process is merely one of foreign body absorption. Soudakewitch regarded the giant cells as phagocytic for elastic fibers, but this Unna[1] opposed, claiming that the giant cells formed around elastic fibers by accident only, the fibers undergoing degeneration by virtue of the same hostile influences that cause degeneration of tuberculous giant cells. As pointed out by Rona, the persistence of elastic fibers within giant cells is the remarkable thing which needs explanation in view of the readiness with which the elastic elements disappear elsewhere in tuberculous and many other inflammatory foci. He suggests that it looks as though the chemical changes in the interior of giant cells were at a standstill. Soudakewitch mentions several older observations concerning elastic fibers in lupus and concerning concretions and vacuolations in giant cells. He also observes that certain giant cells described by Kraus about threads in a carcinoma may have contained elastic fibres.

Among other instances of iron incrustation may be mentioned the ferriferous masses of degenerated tubercle bacilli described by Walker[2] in experimental tuberculosis in the "Zieselmaus;" the siderosis of degenerated ganglion cells described by Weber[3] and by D. J. McCarthy.[4]

---

[1] Histopathology of Skin (transl. by Walker), 1896, 578.

[2] Zeigler's Beitrage, 1895, xviii, 534.

[3] Münch. Med. Wochenschr., 1898 (Phys. Med. Gesellschaft zu Würzburg, May 26, 1898). Monatschr. f. Psychiatr. u. Neurol., 1898, 3.

[4] Contributions from the William Pepper Laboratory of Clinical Medicine, 1900, 107–119.

GIANT CELLS CONTAINING FERRIFEROUS AND CALCAREOUS ELASTIC FIBERS IN A HEALED HEMORRHOIDAL NODULE.

Some months after the excision of hemorrhoids there appeared at the anal margin a small nodule, about one and one-half centimeters in circumference, which was removed and fixed in formalin and alcohol. The sections have an irregularly triangular form and are covered on all sides except the base by a somewhat thickened epidermis. The tissue is fibrous, rich in elastic elements, and contains quite a number of small arteries and venous or lymphatic spaces. There is more or less cellular infiltration everywhere, but in some places this is especially well marked. They are not unlike tubercles with epithelioid and lymphoid cells as well as multinuclear giant cells. The giant cells, however, have not the tuberculous habitus, but correspond to foreign body cells. There are no evidences of necrosis. The giant cells often occur at the periphery of the cellular areas and occasionally in the midst of mature tissue. Many of the giant cells contain peculiarly and variously shaped bodies not unlike some " carcinoma parasites," but which special staining for iron and elastic fibers shows are ferruginous incrustations upon intracellular elastic fragments as well as elastic fibers surrounded by peculiarly shaped mantles, which do not give the reaction for iron. Naturally many curious formations are seen and further descriptive detail is found in the explanation of plate X. In a few instances giant cells are seen to contain or surround the ends of fibers, which stain with acid orcein and with Weigert's stain. There seem to be atypical elastic fibers near some of the cellular foci, but one could not say whether such fibers are newly formed or old. Perhaps the fact that they are rather thick and coarse would speak in favor of their being old fibers. The material did not suffice for carrying out all the microchemical reactions that might be desired. Tubercle bacilli were not found in a number of sections examined for this purpose, but this negative result does not positively exclude tuberculosis in the absence of animal experiments and especially in view of the

fibrous character of the nodule. The question of syphilis must also be considered. Unfortunately this question cannot be determined from the facts at hand. The researches of Federmann[1] and others indicate that the elastic elements may persist in syphilitic lesions while they tend to disappear more promptly in tuberculous foci. But there is probably no justification for regarding the persistence of elastic elements within giant cells in this case as evidence for or against either syphilis or tuberculosis.

SOLUTION OF ELASTIC FIBERS BY EXUDATE AND WITHIN GIANT CELLS IN AN INFLAMMATORY FOCUS.

The tissue in which the changes to be described were observed was obtained from Professor Senn's surgical clinic, constituting the parts removed with excellent cosmetic results from a greatly enlarged and bulbous nose; the diagnosis was rhinophyma. Alcohol and Müller's fluid hardening.

The sections include skin, a number of sebaceous glands, subcutaneous tissue, some muscle, and a few nerves. There are no changes in the epithelium. The papillæ are flattened. There is some leucocytic and embryonal cell infiltration in the corium. The vessels are not thickened; they are filled with well-preserved blood. In the subcutaneous tissue and coalescing with the muscle are three large areas, occupying several low power fields, composed of innumerable multinuclear giant cells, embryonal connective tissue, and polynuclear leucocytes, which in the central parts form dense collections and are interspersed with a granular or fibrinous material. The giant cells are mostly of the type of the foreign body giant cell, the nuclei being massed in the center. Scattered about among the leucocytes and also in the giant cells are numerous, rather prominent and clearly visible, apparently stiff and thick fibers of varying lengths and staining a little redder with eosin than the cell bodies. These pieces of fibers are surrounded generally by a space filled with a faintly granular non-staining material. There are

[1] Virchow's Archiv., 1901, clv, 469–497.

many fibers, but no giant cells, among the purely leucocytic accumulations, which are so dense as to represent more or less distinctly purulent foci. Midway between the centers and the peripheries of the areas giant cells are quite numerous and contain the fibers mentioned. In some places the giant cells seem to flow like plasmodial masses around the fibers; in other cases the fibers lie in vacuoles, either straight, curved, or curled up. Some of the fibers appear uneven, as if partly eaten up or dissolved, often with indefinite margins as if they were softened. There are also giant cells containing empty vacuoles as well as polynuclear cells, the cytoplasm being more or less granular. At the periphery of the areas is embryonal fibrous tissue; here the giant cells are well preserved and mostly free from fibers; some appear to be splitting up into single cells. The appearances briefly outlined in the foregoing are well depicted in Figure 1, Plate XI., a photograph of a smaller area upon the outskirts of one of the larger. Figure 2 and 3 (same plate) show more of the details.

Weigert's elastic fiber stain brings out well the elastic elements of the normal tissue; and so does acid orcein. The elastic elements are abundant, the fibers appearing thick and dense. The majority of the fragments in the cellular areas remain unstained, but many fragments take the specific stains, probably more doing so in the orcein preparations than in the Weigert. Stained and unstained fragments occur side by side, and there are fibers that take the stain only in part, the end remaining unstained. But few of the intracellular fibers are stained typically by these methods, and not any of those in distinct vacuoles.

Potassic ferrocyanide or ferricyanide and hydrochloric acid fail to show any incrustation with iron. Tubercle bacillus stain gives negative results; also Gram's stain for pyogenic cocci. Gram's stain is taken up by the granular exudate in the markedly cellular areas and also by the substance in the vacuoles about the fibers; irregular, roundish, large and small violet areas are scattered about, covering over the fragments. The Gram-Weigert's modification does not give this

stain. In sections stained with orcein and Gram's method brownish fragments of fibers may be seen at one side of some of the violet masses. The possibility that some or all of the fibers described here as more or less altered elastic elements are in reality fragments of keratohyalin and similar substances from disintegrating hornified epithelium is probably excluded definitively on account of the location of the areas, namely in the subcutaneous tissue, the overlying epithelial covering being intact. This fact, together with the positive reaction of the fibres to both the acid orcein and the Weigert staining methods for elastic tissue, seems to me to settle the question. At the same time I am not willing to exclude altogether the possibility that some of the fibers may be of a collagenous nature.

The nature of the lesion in the second case remains obscure. Apparently it concerns an inflammatory focus with exudation and leucocytic immigration, the elastic fibers apparently being in the process of solution. Usually elastic fibers are dissolved readily in acute inflammatory areas, but in this instance solution seems to have been delayed and a very large number of giant cells phagocytic for the elastic fibers have formed. The exact reason for this unusual course cannot be determined by morphologic means; it would seem as if some change in the fibers rendered them more difficult of solution, thus giving them more the character of an ordinary foreign body. Inasmuch as tuberculosis may be excluded quite definitely on histologic grounds, the inference is permitted that the inclusion of elastic fibers and possibly collagenous fibers by giant cells is not peculiar to tuberculosis and similar diseases. The absence of incrustation of the fibers may be explained in part, perhaps, on the score of the relative acuteness of the whole process as indicated by the almost purulent character of the centers of the foci. In the case of the hemorrhoidal nodule, the exact nature of which remains unsettled, the appearances are similar to those described by Rona in tuberculosis of the skin. Here fragments of elastic fibers appear to remain within giant cells for some time and to become incrusted with salts

FIG. 1.

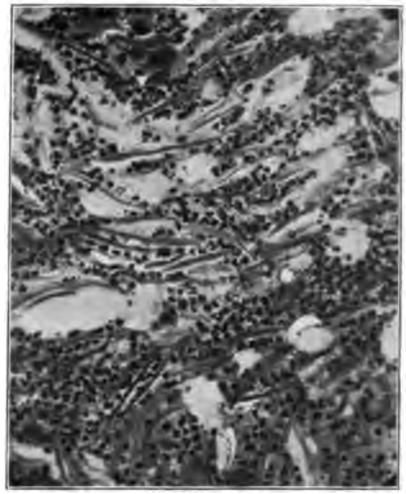


FIG. 2.

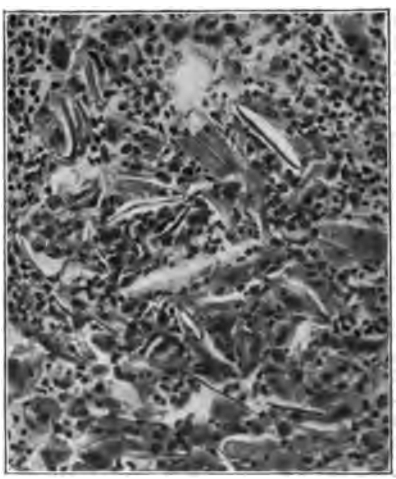


FIG. 3.

FIG. 4.

HEKTOEN. ELASTIC FIBERS IN GIANT CELLS.

FIG. 1.

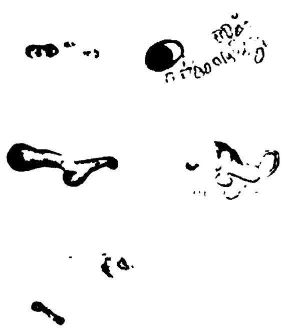


FIG. 2.

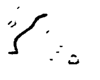


FIG. 3.

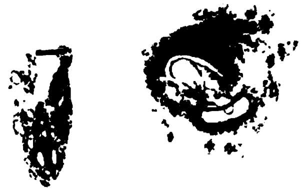


FIG. 4.

FIG. 5.

FIG. 6.

of iron and probably of calcium. Whether the differences of the fate of the elastic fibers in these two cases depend upon differences in the elastic elements or upon differences in the cells and fluids cannot be determined. Either explanation, or both, may be true. In both cases it probably concerns processes of absorption of more or less changed fibers which play the role of foreign bodies.

EXPLANATION OF PLATES.

Plate X.

Fig. 1. — Hematoxylon stain. Multinuclear giant cell with two peculiar concretions in its interior. Note markings of surfaces of concretions. X 800.

Fig. 2. — No nuclear stain, only iron reaction in concretions in giant cells. (Potassic ferrocyanide and hydrochloric acid.) X 700.

Fig. 3. — Giant cell containing a piece of a fiber which gives the reaction for iron. X 700.

Fig. 4. — Acid orcein stain for elastic fibers followed by potassic ferrocyanide and hydrochloric acid. Multinuclear giant cells containing brown fragments (elastic fibers) and concretions that give the reaction for iron. X 800.

Fig. 5. — Same stain as Fig. 4. Elastic fibers surrounded by a peculiarly shaped mantle which does not give the iron reaction. X 800.

Fig 6. — Potassic ferrocyanide and hydrochloric acid followed by Weigert's stain for elastic fibers and by safranin. Shows a peculiar ferruginous concretion, resembling a cross on a rounded pedestal, situated in a giant cell; also elastic fragments among the cells in the upper half of the drawing. Note relations of fibers to the smaller giant cells. X 700.

Plate XI.

Fig. 1. — Small area with leucocytes, elastic fibers, and giant cells; the leucocytes are most numerous in the center, in which there is also some exudate; the plasmodial masses are best marked at the outskirts. X 75.

Fig. 2. — Polymorphonuclear leucocytes, exudate, and elastic fibers. X 200.

Fig. 3. — Elastic fibers in giant cells; many leucocytes scattered about. X 200.

Fig. 4. — Acid orcein stain only; shows irregular staining and failure of many fibers to stain at all.

CHOLESTERIN GIANT-CELLS.

E. R. LeCount.

(*From the Pathological Laboratory of Rush Medical College.*)

The slits or clefts formed by the solution of cholesterin in processes of fixation have been frequently observed in necrotic tissue, granulation tissue, and tumors and their connection with cholesterin crystals recognized. Thoma has pictured these chink-like openings in Figure 12 accompanying his study of Arterio-sclerosis nodosa[1] and although giant-cells occurred in the surrounding granulation tissue they were not supposed by him to be connected with the presence of cholesterin. Hanau[2] has also observed such openings in a melano-sarcoma of the retina, but they were unaccompanied by giant-cell formation.

C. Meyer,[3] in a case of foreign body peritonitis resulting from the rupture into the peritoneal cavity of cystadenoma of the ovary, has described giant-cells due to the presence of cholesterin crystals. The peritoneum was studded with minute nodules that led to the diagnosis of tuberculosis at the operation and induced Meyer to classify the case with those diverse conditions which have for a long time been known under the collective designation " pseudotuberculosis." It is of especial significance that the cyst had at some time previously been the seat of a considerable hemorrhage and that in the large amount of fluid removed by aspiration before the operation (about 5800 ccm.) cholesterin crystals were very abundant. Cells of a regularly oval or elliptical form with a single cleft and thirty or more nuclei were found in the nodules and also cells that had enclosed two or more cholesterin plates.

Incidental to the occurrence of other forms of giant-cells, P. Manasse[4] has described cholesterin giant-cells in polypous

---

1 Virchow's Archiv., 1886, cv, 1.
2 Quoted by Meyer.
3 Ziegler's Beiträge, 1893, xiii, 76.
4 Virchow's Archiv., 1894, cxxxvi, 245.

growths of the middle ear accompanying chronic suppuration. In some of them the crystals were present. Crämer and Schultze [1] have noticed them in a tumor of the eye, and Ruge [2] and Krückmann [3] have described their occurrence in sebaceous cysts.

Becher,[4] who has written quite extensively upon the occurrence of giant-cells in rather slowly growing carcinomas — the so-called cancroids — describes in one case giant-cells that he thought might be due to cholesterin or to medicaments, but he was uncertain which was the factor.

Although the occurrence of openings due to cholesterin and the giant-cells that form around them are fairly well known to those whose opportunities entail the routine examination of pathological tissues, there are, with the exception of the above references, few allusions to them in literature, and save for those accompanying the article of Meyer, few good illustrations.

CHOLESTERIN GIANT-CELLS IN OLD SCROTAL LESION.

I received from Dr. Hunter a testicle and tumor-like mass removed by him from a man who two years previously had suffered from an acute orchitis with effusion into the tunica vaginalis. This was removed by aspiration, but there remained a hard mass behind the testicle, and the epididimis and cord were enlarged. The testicle was painful, and suspecting tumor or tuberculosis the organ was removed.

Macroscopic Examination. — The specimen is the size of a man's fist, weighs nearly half a pound, and is quite solid. The upper part is rough and covered by loose fibrous tissue. The lower portion is quite smooth in front, rough and shaggy behind. It is eleven centimeters high, nine centimeters transversely above, and five centimeters below, and six centimeters from before backward. On cutting the tumor through from before backward the testicle is found embedded

[1] Archiv. für Augenheilkunde, 1894, xxiii, 293.
[2] Virchow's Archiv., 1894, cxxxvi, 523.
[3] Ibid., 1895, cxxxviii, Supplementheft, 118.
[4] Ibid., 1899, clvi, 62.

in a mass of fibrous tissue; it is of normal size, but appears flattened. Directly behind the testicle is a band of firm fibrous tissue one centimeter thick, which extends vertically the entire length of the organ, and above it, curves backward. Posterior to this and opposite the middle portion of the testicle is an oval body with its long axis vertical; it measures three centimeters in length, and lies rather loosely in the surrounding tissue. Its greatest width is one and one-half centimeters, and when lifted from the bed in which it lies it is seen to possess a shining capsule. Above the testicle is a mass of firm tissue which extends more toward the left. On the lower part of the specimen and on the right side, about six centimeters of thickened cord is visible.

Microscopic Examination. — Sections were studied from the entire surface. The interest centers about those sections which came from the oval body behind the testicle. This is formed almost entirely by groups of plate-like or sheet-shaped giant-cells. The groups are oblong or elliptical in shape, and the giant-cells in each group are arranged quite parallel to one another. These plate-like giant-cells arranged side by side when sectioned have an appearance of elongated fibers which at one time contained cholesterin tablets or crystals. In certain groups the section has not been at right angles to the plane of the sheet and the fiber-like giant-cell appears wider in consequence; still rarer are sections which show the flat side of the sheet-shaped giant-cells. These groups are separated from one another by rather old granulation tissue in which the vessels are both numerous and thin walled. Considering more carefully the giant-cells as seen in serial sections, certain differences in the degree of development are noticeable. Isolated giant-cells are found in the above-mentioned granulation tissue; they contain only a single cleft, the solitary cholesterin tablet having been dissolved by the alcohol. Such giant-cells are elliptical in shape; the nuclei are numerous and located close to the cleft in some cells; for the main part, however, they are clustered near the periphery. As the giant-cells become larger so as to enclose two cholesterin plates, there are two long and narrow

clefts which often diverge; there results a pyramidal giant-cell between the clefts which may contain twenty or more nuclei in a single section. In serial sections such small giant-cells enclosing one or two cholesterin crystals may be followed in fifteen or twenty sections, consequently the number of nuclei which they possess may run into the hundreds. From these giant-cells there are gradual transitions to the large forms which encapsulate many parallel cholesterin tablets, and which appear in the sections as long fiber-like cells with many nuclei. Such extreme types occur only in clusters that are referred to above as oblong or elliptical in shape. The giant-cells of many of these groups have become broken and distorted in the various stages of hardening, sectioning, etc., so that holes occur into which the slender and torn giant-cells project. The nuclei of these cells are small and stain faintly. In the vascular tissue between the groups are occasional cells, with small nuclei staining very darkly, which from their shape and location about the vessels are undoubtedly leucocytes.

After finding these appearances, the alcohol in which the specimen had been preserved was examined for cholesterin as follows: The alcohol was evaporated carefully by allowing it to fall drop by drop upon heated sand. The object of thus carefully evaporating the alcohol was to prevent decomposition of the cholesterin. The sand, which had been previously incinerated and purified, was then shaken with chloroform and allowed to stand over night to obtain, if possible, the cholesterin in solution. This was then subjected to the following tests for cholesterin:

1. If strong sulphuric acid be added to the chloroform solution a play of colors is noticed, violet usually predominating. On exposure to air the color changes to green, and a marked fluorescence is noticed.

2. If some of the substance remaining after evaporating the chloroform be evaporated with nitric acid, a yellow mass, turning brick red on addition of ammonia hydrat, is noticed.

3. If the solid substance be evaporated with a mixture of

two volumes of sulphuric acid and one volume of ferric chlorid, a violet color is pródouced.

Cholesterin was found by all of these tests; the first test is considered quite characteristic.

CHOLESTERIN GIANT-CELLS IN CARCINOMA OF SCALP.

In sections from a carcinoma which made its appearance near the mastoid process and invaded the occipito-frontalis fascia and the hair-bearing scalp, attaining the size of a goose egg, there were found giant-cells in many respects similar to those described in the mass behind the testicle. The tumor for the most part consists of epithelium arranged in masses of rounded contour; the stroma is very scanty and rarely exhibits any inflammatory changes. In certain sections there are aggregations of cholesterin giant-cells contained in a tissue which evidently represents some retrogressive change in those epithelial cells which correspond to the outermost layers of stratified epithelium, those farthest from the corium, in the tumor farthest from the stroma. Amongst these giant-cells, and between the groups of them, are leucocytes and other cells similar to those ordinarily found in granulation tissue; furthermore, delicate thin-walled blood vessels occur here whose presence is difficult to understand until it is noticed that this tissue on one side borders on or is connected with the stroma of the tumor. In some sections this connection is not shown. The giant-cells do not form so large plates as those in the scrotal process; the greater width of the cleft is perhaps due to a shrinkage in alcohol, but here, as in the scrotal process, the cholesterin tablets were accumulated side by side, as one would place photographic negatives in a plate holder, and the giant-cells grew between and encapsulated them. Thus were formed, here and there, bunches or groups of giant-cells, which being sectioned, appear like multinucleated delicate fibers.

EXPERIMENTAL CHOLESTERIN GIANT-CELLS.

Cholesterin crystals (Merck) added to physiological salt solution and sterilized by fractional distillation with steam

were injected subcutaneously into the back of a guinea-pig. Injections were made in various places and one of the resulting nodules was removed at the end of eleven days.

Sections of this nodule show the central content of necrotic tissue staining darkly with hematoxylin with a few pointed sharp clefts marking the site of former cholesterin plates. The surrounding wall is formed mainly by giant-cells that vary greatly in size, the larger ones occurring in the middle and outer zones. This granulation tissue is very vascular, and the vessels are all thin walled and filled with blood. The inner part of the wall contains a few small collections of red blood corpuscles lying free in the tissue. Adjacent to the necrotic tissue the cholesterin crystals evidently became arranged radially, since very small clefts occur between spindle-shaped cells that point toward the necrotic tissue; these cells possess flattened nuclei that stain lightly. A little outward the clefts between the cells are closed in as though the fibrillating cells had coalesced so as to encapsulate the crystal. Such giant-cells enclosing a single crystal may possess simply two nuclei, or the cleft from which the cholesterin has been dissolved may divide four nuclei into pairs. The larger giant-cells consist for the main part of nuclei, as many as twenty, thirty, or more being present in a single thin paraffin section; yet other giant-cells consist largely of body protoplasm. When these large cells possess many clefts such openings are usually parallel or nearly so. The chromatin of the nuclei of these cells usually borders the inside of the nuclear membrane. Many giant-cells contain leucocytes in divers degrees of destruction, as is shown by the nuclear chromatolysis. When, in fortunate sections, these giant-cells are viewed from the side, the space formerly occupied by the cholesterin tablet is seen to be irregular; the form taken by cholesterin in pathological fluids of a square or notched square does not seem to have been retained in these plates.

Cholesterin suspended in bouillon was, with the assistance of Dr. Brown Pusey, injected with a sterile pipette into the anterior chamber of the eye of a rabbit. During the opera-

tion the ciliary body was entered and a prolapse of a small part of the iris occurred. This eye was removed after ten days.

Histologically, giant-cells similar to those described in the other lesions were found not only in the ciliary body and the corneo-scleral junction, but also in the track of the incision through which the pipette entered. The wound healed without infection, and the site of the incision is found to be made up of granulation tissue composed of fibrillated cells with a number of newly-formed blood vessels. Here, however, most of the giant-cells bear pigment; the genesis of these cells is evidently in the pigmented cells of the ciliary body. The very small clefts are bordered by two small, slightly pigmented and fibrillated cells; the larger clefts are enclosed by longer fiber-like cells on either side. These, as in the other instances described, show a pyramidal end where they join the adjacent tissue.

PLATE XII.

FIG. 1. — Cholesterin giant-cells. One end of a clump showing the pyramidal bases where the giant-cells are continuous with the adjacent tissue. Above the center some of the sheet-like giant-cells have been cut obliquely.

FIG. 2. — Cholesterin giant-cells. From the case of periorchitis and organized hematocele. A single giant-cell encapsulating a small plate. Such small cholesterin giant-cells are the forms that have been generally described.

FIG. 3. — Form of cholesterin giant-cell obtained experimentally in the subcutaneous tissue of a guinea-pig.

FIG. 1.

FIG. 2.

FIG. 3.

LECOUNT.

CHOLESTERIN GIANT-CELLS.

ON THE LEUCOCYTES OF THE CIRCULATING BLOOD OF THE RABBIT.*

WALTER R. BRINCKERHOFF, M.D., AND E. E. TYZZER, A.M.

(From the Pathological Laboratory of the Boston City Hospital and the Sears Laboratory of Pathology of the Harvard Medical School.)

The following report comprises the results of a comparative study of the pictures presented by the leucocytes of the rabbit in (1) dried smears of blood, fixed by heat and stained with the usual blood stains, (2) blood smears treated by Scott's wet method, (3) sections of fixed tissue stained with the solutions usually employed in pathological work.

The only systematic statement of the morphology of the leucocytes of the rabbit, of which we are aware, is that of Hirschfeld.[1] He studied dried blood smears from various mammals and gives an excellent account of the leucocytes of the rabbit so far as revealed by the technical methods which he employed.

In the accounts of researches dealing with various problems as to leucocytes, leucocytosis, inflammation, and the like, there are scattered references to the morphology of the leucocytes of the rabbit, but always as side issues to some problem more immediately at hand. For example, in the work of Ehrlich and his pupils, of Arnold, Jolly, Muir, Gulland, Kanthack and Hardy, and a host of others. As the titles of such articles rarely suggest that observations upon the morphology of the leucocytes of the rabbit are to be found in them, the compilation of a complete bibliography upon the subject of this article is practically impossible. We have therefore had to depend for knowledge of previous observations upon such statements as could be gleaned in general reading of original researches upon experimental leucocytosis, and the like.

We believe that a sufficient justification for this attempt

* The expenses of this research were paid from a Bullard Fellowship.

at a systematic description of the leucocytes of the rabbit is given by the occasional blunders which mar the account of otherwise excellent researches upon experimental leucocytosis in that animal. Muir[2] cites a case in which the amphophiles were confounded with the eosinophiles; and we have encountered an instance in which neutrophilic leucocytes were enumerated in rabbit's blood.

The nomenclature of Ehrlich will be followed, synonyms being given where known. No attempt will be made to describe the finest details, but rather stress will be laid upon those grosser structures of the cell which serve for identification.

What we believe to be new in this work is the homologising of the picture presented by the leucocytes in blood smears with that in stained sections of fixed tissue.

GENERAL MORPHOLOGY OF LEUCOCYTES OF RABBIT.

The two criteria which have proved of greatest service in classifying leucocytes are the morphology of the nucleus and the micro-chemical reactions of the protoplasmic granules. The thoroughness of the latter method of classification is still in dispute; and it seems best to follow the plan of Hirschfeld, making the morphology of the nucleus the first basis of separation into classes, reserving the micro-chemical reactions for further subdivision.

Following out this plan the leucocytes of the rabbit, as seen in blood smears, are primarily divisible into two groups on the basis of nuclear morphology, viz.:

I. *Mononuclear leucocytes*, in which the nucleus is in a single rather symmetrical mass.

II. *Polymorphonuclear*, or better, *polynuclear leucocytes*, in which the nucleus is in several masses which are connected by relatively slender bridges of nuclear material.

The mononuclear leucocytes comprise two types of cells: in one the protoplasm (under certain treatment) has a strong, in the other a weak, affinity for basic stains. The

protoplasm contains no true granules. These are the lymphocytes and the large mononuclears respectively.

The polynuclear leucocytes are divisible into three types on the basis of cell granules. In one type the granules have an affinity for the basic stains, in another a selective affinity for certain acid stains, and in the last a constant affinity for eosin. These are the mast cells, the amphophiles, and the eosinophiles respectively.

All these attributes of the leucocytes of the rabbit are demonstrable in dried smears from the circulating blood. On these criteria is based the classification of the leucocytes generally used in hematology,* as follows:

I. Mononuclear (non-granular) leucocytes.
  1. Lymphocytes.
  2. Large mononuclears.

II. Polynuclear (granular) leucocytes.
  1. Amphophiles.
  2. Eosinophiles.
  3. Mast cells.

TECHNIQUE.

Dried Blood Smears. — Thin films of blood were made upon slides or cover glasses and allowed to dry in the air. The method of making the spreads was as follows: A small drop of fresh blood is touched with the edge of a clean cover. The edge of this cover, with the drop of blood adhering to it, is pressed against the surface of a second cover glass, the two making an angle of about forty-five degrees. Capillary action spreads the drop of blood along the angle formed by the two glass surfaces, and the blood is then evenly distributed on the second cover by sweeping the first across its surface.† After the smears were thoroughly dried they were put in an oven and baked for a half hour at a temperature of from 110° to 120° C. Various other methods of fixation

* This classification is essentially that of Ehrlich.

† This method of smearing blood has proved more serviceable than the better known method of Ehrlich.

were tried; but as the above method is that most generally used in blood work, the descriptions which are to follow will be based upon preparations so treated. The smears were stained with Ehrlich's tri-acid mixture, Ehrlich-Biondi-Heidenhain three-color mixture, and with aqueous solutions of various acid and basic anilin dyes, both alone and in combination. (Methylene blue, methyl green, thionin, eosin, aurantia, indulin, etc.)

Blood Smears by Scott's Method.[3] — We have found this method very valuable in that it presents a view of the leucocytes intermediate between that seen in dried smears and in sections of fixed tissue. In brief his technique is as follows: (1.) Make a rather thick spread and hold it for five seconds, wet side down, in the mouth of a wide-necked bottle which is half filled with forty per cent formaldehyde. (2.) Drop, while still wet, into absolute alcohol. The film is to remain in this fluid for at least fifteen minutes. No harm will follow if it is left in alcohol for forty-eight hours. (3.) Blot off the excess of alcohol and move to a dry part of the blotter. (4.) Immediately, before drying takes place, put on a few drops of Jenner's stain with a pipette. Cover with a watch glass to prevent evaporation and consequent precipitation of the stain. Stain for two minutes, *not longer*. (5.) Allow the excess of stain to run off and rinse in two changes of distilled water. (6.) Blot off the excess of water. (7.) Dehydrate by dipping once into absolute alcohol. This step must be done very rapidly. (8.) Wash off alcohol at once in at least three changes of xylol. (9.) Mount in balsam.

Sections of Fixed Tissues. — The leucocytes were studied in various tissues as found in the vessels, in extravasations, and in exudates. The material was from freshly-killed adult rabbits, and was preserved in alcohol, Zenker's fluid, formaldehyde (four per cent aqueous solution), saturated solution of corrosive sublimate in four per cent formaldehyde, and saturated solution of sublimate in normal saline solution. The tissues were imbedded in paraffin by the chloroform method, and sections three to six microns in thickness were cut with the Minot microtome. The stains most used were Unna's

Methylene blue-eosin and Mayer's Alum Hematoxylin or Ehrlich's Acid Hematoxylin followed by Eosin. Other stains, as Mallory's Iron Hematoxylin, etc., were tried, but as the first-mentioned stains are most commonly used in pathological work, only the leucocyte picture will be considered which those stains show.

I. MONONUCLEAR, NON-GRANULAR LEUCOCYTES.

1. *Lymphocytes.* — The details of nuclear and protoplasmic structure in the lymphocyte are brought out with different degrees of clearness with different methods of staining and fixing. Protoplasmic pictures are most complex in dried smears fixed with heat and stained with a simple basic dye. On the other hand, the nucleus yields more apparent structure in sections of fixed tissue and in smears treated by Scott's wet method.

General Morphology. — Cells of about the size of red blood corpuscles, having an approximately circular outline and containing an almost circular nucleus surrounded by a relatively narrow zone of protoplasm.

Dry smears, Ehrlich's tri-acid mixture. Protoplasm a faint pink, often leaving unstained a narrow, sometimes crescentic, zone about the nucleus. Nucleus a grayish-blue or slate color. The nucleus is frequently traversed by a network of a deeper shade which suggests wrinkles in a stained nuclear membrane.

Methylene blue. The nucleus stains blue and is partly obscured by the deeply stained reticular structure of the protoplasm. Nodal points, often of rather large size, are frequently present in the protoplasm. These pseudo-granules are irregular in size and shape and are not to be compared with the regular, evenly contoured, protoplasmic structures which occur in the true granular leucocytes. If the smear be differentiated with a dilute solution of acetic acid the cyto-reticulum, in many cells, retains the stain after the nucleus is almost completely decolorized.[4]

Smears by Scott's method. The lymphocytes present the same appearance as in sections of fixed tissue, stained with

Unna's Methylene blue and Eosin, with the exception that the protoplasm seems more abundant and structureless. This apparent increase of protoplasm is, no doubt, due in part to the cells being spread upon a plane surface instead of being fixed while suspended in a fluid.

Sections of fixed tissue. Unna's Methylene blue and Eosin. The protoplasm is very scanty and may even seem absent. If the blue stain predominate a fine protoplasmic reticulum can sometimes be made out.

If the section be stained deeply with Eosin, the protoplasm is homogeneous and takes the red stain. In blood vessels and in exudates, the nucleus, as a rule, shows very definite chromatin masses which are large, sharply circumscribed, and appear as though plastered against the inside of the nuclear membrane. The most common appearance is four to six irregularly triangular masses of chromatin arranged about the periphery of the nucleus with one side of each mass applied to the nuclear outline. The two free sides of the mass form an obtuse angle directed toward the center. An irregular mass of chromatin usually occupies the central portion of the nuclear area. From this typical adult lymphocyte every gradation is to be found — to a cell slightly larger in size but having a nucleus of the vesicular type, *i.e.*, the chromatin being in granules and threads. These latter cells are identical with the predominant cells of the lymph nodes.* In sections stained with Hematoxylin and Eosin the nuclear picture is the same as above described. The protoplasm, however, does not show a reticulum.

Councilman[5] has mentioned this characteristic appearance of the nucleus of the lymphocyte of the rabbit.

Atypical Lymphocytes. — Indented nucleus. The indentation of the nucleus of lymphocytes, which is occasionally seen in blood smears, is usually in relation to a point in the nuclear outline where chromatin masses are wanting. It seems probable that the indentation is due to a falling in of

* Taylor [6] has expressed this view with regard to human blood. Histologists generally hold the opinion that the circulating lymphocyte is derived from the cells of the lymph node "germ centers."

the nuclear membrane at a point where there are no relatively dense masses of chromatin to give support. Such an infolding of the nuclear membrane might well follow the loss of fluid from the nucleus. This seems a reasonable explanation of the nuclear nick in the lymphocytes. Its only importance is as a possible beginning of polymorphism of the nucleus of this cell. If the depression were of that nature, further variations of form should occur. We have found none such. Intermediate forms are wanting to connect this lymphocyte having an indented nucleus with the large mononuclear leucocyte with a "saddle-bag" or "horse-shoe" nucleus. In rabbit's blood lymphocytes with the marked indentations such as are seen in human blood in certain leukemias are not to be found. (4.)

Double nuclei. In two instances we have found cells in blood smears which were undoubtedly lymphocytes, but which had two semi-circular nuclei placed with their straight sides almost in apposition. In one of these cells there was a considerable disparity in the size of the two nuclei, in the other they were almost equal.

2. *Large Mononuclear Leucocytes.** This cell has played an important part in the controversy which has been waged over the inter-relationship of the leucocyte forms. In schemes wherein the leucocytes are regarded as stages in the metamorphosis of a single cell, this form figures as the bond between the polymorphonuclear and the mononuclear types. For the present we will be content with a simple description of the cell as it appears after staining by the methods under comparison.

General Morphology. — A cell of about twice the diameter of the red corpuscle, having an oval, elongated, or curved nucleus and whose protoplasm has a slight basic affinity, but contains no true granules. By certain methods of staining and in the fresh state (Kanthack and Hardy) the protoplasm presents the so-called "ground glass" appearance. This

*Mononuclear amœboid cells (Max Schultz), Hyaline cell (Kanthack and Hardy), Non-granular leucocyte (Councilman), Large mononuclear and transitional forms (Ehrlich), Ungranulated mononuclear cells (Taylor), etc., etc.

phenomenon is supposed to be due to the presence of minute granulations,[7] but lacking the well-defined structural elements which are found in the protoplasm of certain other leucocytes, *e.g.*, amphophiles, eosinophiles, etc., is termed accordingly non-granular.

Dried smears, fixed by heat, Ehrlich's Tri-acid stain. The nucleus shows no definite structure and takes a diffuse faint blue stain. The protoplasm stains faintly pink. With methylene blue staining the protoplasm shows an affinity for the stain, though it is less than in the lymphocyte.

Smears by Scott's method. The nucleus contains a delicate network of chromatin threads. We have been unable to convince ourselves of the presence of true nucleoli as described by Kanthack and Hardy. The protoplasm has the "ground glass" [7] appearance above mentioned and in this respect the cell is in sharp contrast with the lymphocyte, which, under the same treatment, shows no protoplasmic structure.

Sections of tissue, Zenker's fluid, Unna's Methylene blue and Eosin. The nucleus stains faintly and contains a delicate chromatin network.[7] The nucleus is never divided into masses, though it frequently has a definite horse-shoe shape.[5] The protoplasm does not stain very intensely and is translucent rather than transparent. No protoplasmic granules are present, though under favorable circumstances a cyto-reticulum with nodal points can be made out. Durham[8] mentions hyaline cells with two nuclei which he found in peritoneal fluid.

II. POLYMORPHONUCLEAR (GRANULAR) LEUCOCYTES.

1. *Amphophiles.** — Dried smears. Fixed with heat.

---

* This cell is the equivalent of the neutrophile leucocyte of human blood. According to Jolly[9] the term "amphophile" was first applied to the cell by Schwartze, who was a pupil of Ehrlich. The term has received the sanction of Ehrlich and is used by him in his latest work on blood.[4] According to Ehrlich's classification of leucocytes the cell is characterized by its possession of a granule which takes either the acid or basic stain. The term "amphophile" is therefore a descriptive one like "neutrophile." Certain observers, as Kanthack and Hardy, Jolly, and Durham, specifically state that they could not stain the granules with a basic dye. As will be noticed later, we agree

Stained with Ehrlich's Tri-acid stain. The nucleus takes an uneven blue color. No definite nuclear structure can be made out and the nuclear bridges uniting the beads can seldom be seen. The nucleus is closely surrounded by a mass of small, bright red staining granules, which are distinctly oval in shape. Hirschfeld [1] speaks of these granules as being spherical. We cannot agree with this statement. There is not such a marked disparity between the axes of the granule as in the eosinophile, but to us they are by no means spherical. The inter-granular protoplasm is not stained. All the cells contain approximately the same number of granules. That is to say, there are no cells with notably few granules. Jolly [9] speaks of finding polymorphonuclear leucocytes without granules, others filled with granules, and between these two extremes all intermedlate forms. With this statement we cannot agree. We have not found truly polymorphonuclear leucocytes in the blood of the rabbit which did not contain either basic or oxyphilic granules.

The nuclear outline is not modified by the granules. As will be noted later, this is in contrast with the molding of the nucleus of the eosinophiles.

Methylene blue. The nucleus as a whole stains deeply and in a majority of the cells shows a definite massing of the chromatin material. The appearance is as though the chromatin was in dense masses lying against the inside of the nuclear membrane, the balance of the nuclear bulk being made up of non-staining substance. Kanthack and Hardy speak of a fine network of chromatin in the nucleus of the amphophile. To us the arrangement of the chromatin of the nu-

with these authors. On the other hand, Hirschfeld and Muir use the term "amphophile" and state that the granules do take the basic stain.

The contradictory statements about this granule may rest upon species variation. Schreiber and Newmann [10] have shown that different species of rabbits show different tinctorial reactions as to their mast cell granules. A similar difference may exist in the amphophiles.

The cell has received other names, as "fine granular oxyphile" (Kanthack and Hardy), "Microxycyte" (Durham), "Pseudo-eosinophile" (used by Rubinstein), "Oxyphile" (Gulland).

In this article we have used Ehrlich's term "amphophile," despite the fact that we have no evidence to show its true descriptiveness.

cleus of the amphophile is comparable with that in the small (adult) lymphocyte, *i.e.*, in definite masses closely applied to the nuclear membrane. The protoplasm is studied with very fine and irregular blue or violet granulations which suggest the nodal points of a cyto-reticulum. We have been unable, after repeated trials, to stain the specific granules of the cell with a basic anilin dye.* (Methylene blue, Thionin, etc.)

Mayer's Alum Hematoxylin and Eosin. We have frequently noted the failure of the granules of the amphophile to stain with Eosin after the use of Hematoxylin. This phenomenon has been mentioned by Schwartze, Dominici,[11] Jolly,[9] Muir,[2] and others. Jolly states that by certain manipulations the granules can be made to stain. Muir mentions the reaction as a useful one in distinguishing between the amphophile and the eosinophile, as smears stained with hematoxylin and eosin show the amphophile with a rather even red protoplasm, while the granules of the eosinophile are sharply differentiated.

Eosin-aurantia-indulin. Hirschfeld records the fact that in dried smears fixed by heat the amphophile granules selected indulin in a mixture of eosin, aurantia, and indulin. Jolly [9] says this selective affinity depends upon the time and degree of heat used in fixation.

Wet preparations by Scott's method. The nuclei show definite sharply circumscribed masses of chromatin arranged as described above. There is a space about the nucleus which is free from granules. The inter-granular protoplasm is not colored and the granules take the eosin stain. The outer limit of the cell is visible as a delicate pink line which is separated from the granules by a narrow clear zone. The granules do not show the slightest tendency to take the basic stain.

Sections of fixed tissues. The picture which the cell presents is governed to a large extent by the fixative used. The nucleus presents a fairly uniform appearance, but the characteristic protoplasmic granules show variations in size and

* The rabbits employed were a cross between the so-called Belgian hare and the common domestic rabbit.

staining capacity after treatment with different fixing fluids.

So far as we have observed, the granules always take the acid dye. We have been unable to demonstrate a selective affinity of the granule for Indulin in fixed tissues. Formol preserves the granule well, and it then appears larger than after any other of the fixatives which we have used. The affinity of the granule for Eosin seems to be increased by the action of formol. Arnold [12] recommends this fixative for cell granules in the marrow. Corrosive sublimate preserves the granules well and increases their affinity for acid dyes (Kanthack and Hardy). The same authors state that immunizing doses of toxines cause an increase in the refractiveness and oxyphilic affinity of these granules. Alcohol (80 per cent) does not preserve the granules at all well, and after such fixation it is frequently difficult or impossible to demonstrate their presence.

Zenker's Fluid. This fixative preserves the amphophile granules well. If care be taken to cut the material in thin pieces the leucocyte picture which can be had with Unna's Methylene blue and Eosin staining is unexcelled. If the material is in thick pieces the granular picture in the deeper parts is lost, presumably from the unequal penetration of the constituents of the fluid. In Zenker-fixed material the granules stain well with Eosin after Hematoxylin. For some reason the granules appear smaller in such preparations.

In inflamed mesenteries the amphophiles of the exudate show a very definite cell membrane which is frequently separated by a clear zone from the granules. Councilman [5] states that the cell has the same appearance in inflamed corneas stained with Mallory's Phosphotungstic-acid-hematoxylin.

2. *Eosinophiles.** — A cell somewhat larger than the amphophile, having a polymorphous or moniliform nucleus and characterized by the presence in its protoplasm of large bluntly fusiform granules which have a strong affinity for Eosin.

* Coarse granular oxyphile (Kanthack and Hardy), Megoxycyte (Durham), Eosinophile (Ehrlich and the majority of authors).

Dried smears fixed by heat. Ehrlich's Tri-acid stain. The nucleus is in two, three, rarely four masses connected by relatively fine threads of nuclear material. The nucleus takes rather a faint blue stain, showing less affinity for the dye than the nucleus of the amphophile. No definite nuclear structure can be made out. The nucleus is surrounded by a number of large red staining granules which have a distinctly oval shape and bluntly pointed extremities. The inter-granular protoplasm does not stain.

Methylene blue. The nucleus takes the stain quite deeply, but not with the intensity of the nucleus of the amphophile. Chromatic masses are distinguishable, though vague in outline. The granules do not stain, but the inter-granular protoplasm takes a bluish tint. The nuclear outline is scalloped, the depressions corresponding to the adjacent granules. This molding of the nucleus by the granules is without doubt an artifact due to drying, as it is not seen in wet preparations or in fixed tissue. Under similar treatment the nucleus of the amphophile remains evenly contoured. The outline of the cell is not even, but is made up of a series of projections and hollows. The projections are formed by the granules, while the intervals are only partially filled by the inter-granular protoplasm.

Wet preparation by Scott's method. The nucleus shows definite masses of chromatin substance which are murally arranged in the beads of the nucleus. The granules stain red and the inter-granular protoplasm is not colored.

Sections of fixed tissue. The picture which the eosinophile cell presents is much less dependent upon the fixation than is the amphophile. Practically all the fixing fluids preserve the granules well and the characteristics of the cell are so marked that its identification is easy. Where confusion might arise between the eosinophile and the amphophile, the differentiation is facilitated by the use of alcohol for fixation. (Cf. p. 19.) The nuclear picture varies in different situations. In the bone marrow the nucleus may be horse-shoe shaped and vesicular. Even when the nucleus is lobed it may have its chromatin in granules and threads. In the cir-

culating blood, in the sub-mucosa, and in exudates the nuclear beads show, as a rule, a definite aggregating of the chromatin substance, the masses having a mural arrangement. At times the nuclear beads may appear very like the nuclei of adult lymphocytes though much smaller. This appearance is well shown in the eosinophiles of the sub-mucosa of the intestine.

3. *Mast Cells.* — We speak with some diffidence about this cell, as we feel that we know little about its occurrence outside the circulating blood. Kanthack and Hardy[7] speak of two sorts of wandering cells of the rabbit which have basophilic granules. These are the coarse granular basophile found in the peritoneal fluid and the fine granular basophile found in the circulating blood. As to the first of these cells, which Kanthack and Hardy describe as explosive on account of their unstable nature, we must plead ignorance. As the cell is not found in the circulating blood we have made no effort to study it. The fine granular basophile of Kanthack and Hardy or the Mast cell of Ehrlich is here described. The cell is numerically of importance in rabbit's blood. Hirschfeld speaks of its being numerous in all the animals which he examined. We have found the mast cells present in such numbers as to comprise five per cent of the leucocytes.*

This is in contrast with the rare occurrence of the analogous cell in human blood.

Dried smears fixed with heat. Ehrlich's tri-acid stain. The nucleus stains faintly and diffusely blue. In its affinity for basic dyes the nucleus of the mast cell is comparable with that of the large mononuclear. The nucleus is polymorphous or moniliform in shape. The beads of the nucleus are relatively large, rather round, and merge abruptly into the fine threads of connecting nuclear material. The protoplasm does not stain and does not have the refractile appearance which characterizes the cell in human blood. As shown by

* The relation of the Mast cell to the clasmatocyte of Ranvier has been fully treated by Schrieber and Newmann (*loco cit.*). We are concerned here only with its morphology as seen in blood smears. The variation in the numbers of this cell in different species of rabbits, as shown by these authors, no doubt explains the scant attention which the cell has received from many investigators.

Ehrlich's stain, this cell might easily pass for a polymorphonuclear form of the large mononuclear leucocyte.

Methylene blue. The nucleus stains faintly and diffusely. The protoplasm is studded with small spherical granules which take the stain meta-chromatically. The granules are slightly irregular in size and are not evenly distributed in the cell.

Wet preparations by Scott's method. The appearance is very similar to that shown by the last described.

STATISTICS.

The average of a large number of differential counts of the blood of normal rabbits gives the following percentage of the leucocyte forms:

Lymphocytes . . .	45 to 55 per cent.
Large mononuclears . .	2 to 8 per cent.
Amphophiles . . .	40 to 50 per cent.
Eosinophiles . . .	0.5 to 1 per cent.
Mast cells	4 to 8 per cent.

THE RELATIONSHIPS OF THE LEUCOCYTE FORMS.

With the refutation of the earlier theories as to leucocyte relationship which regarded leucocytes as the stages in the metamorphosis of a single cell, and with the general acceptance of the fact that the leucocytes are morphologic units, the way has been partly smoothed for a genetic classification. That such a classification would be even now premature is evident from the fact that the origin of certain of the leucocytes still remains in doubt.

Recent morphological and particularly experimental studies have shown, beyond a reasonable doubt, that the bone marrow is the locus of the origin of the amphophiles and eosinophilic leucocytes.* There is strong, though not conclusive, evidence that the mast cells of the circulation have the same place of origin. The lymphocytes have long been known to

* We will discuss this subject in another article.

come from the germ centers of the lymph nodes. There remain the large mononuclear leucocytes.

While the origin of the two cells, the mast cell and large mononuclear, is in dispute, a fully genetic classification cannot be made, further than to group all the leucocytes together as highly differentiated mesenchymal cells.

Even when the amphophiles and the eosinophiles alone are considered it is not fully settled from what cells they primarily arise. Although the amphophile leucocyte is generally admitted to arise from the amphophilic myelocyte and the eosinophilic leucocyte from the eosinophilic myelocyte, the antecedent cells of these are in question.

With regard to the origin of the large mononuclear the following observations are of interest:

Councilman, Mallory, and Pearce [13] in their monograph on diphtheria state their belief that the large mononuclear cells found normally in the lymph sinuses of lymph nodes, in the lymphatics and blood vessels of the lung in pneumonia, and in the lymphatic system and circulation generally in typhoid fever are the large mononuclear leucocytes of the writers on blood. They further state that they consider these cells as endothelial and as having their origin from the endothelium of the lymph and blood vessels. They deny that these cells undergo a transformation into neutrophilic leucocytes.

From our study of the cells in the rabbit, both under normal and under experimental conditions, we have been led to accept this view. In the rabbit the cells seem to have the same morphological and functional characteristics.

The mononuclear cells in the exudates of pneumonia described by Pratt [14] have probably a double source. Some undoubtedly arise from the epithelial lining of the air spaces and some by migration from the vessels.

SUMMARY.

Our study has brought home to us the truth of Taylor's [6] remark about Ehrlich's triple stain. "Advantageous as this stain is for the demonstration of the so-called neutrophilic granulations, it is very poor for nuclei and most pro-

toplasmic structures." For the study of rabbits' blood we have found that Jenner's stain, as in Scott's method or on dried smears, gives far superior results for cell study. For one who is familiar with the leucocytes as seen in sections of fixed tissue, this double anilin stain is of great assistance in studying blood smears.

In the rabbit the disposition of the chromatin substance of the nucleus of the lymphocytes, amphophiles, and eosinophiles suggests somewhat the picture presented by degenerate corneal corpuscles, described by Councilman,[5] which are undergoing direct division, viz.: the chromatin is in masses arranged about the periphery of the nucleus. In the leucocytes this condition can be traced from an antecedent vesicular state of the nucleus. While we do not wish to revive the view that the nuclear form of the polymorphonuclear leucocyte is a sign of degeneration, we do regard it as a sign of specialization of the cell.

It has come to be accepted that the leucocytes do not as a rule divide by mitosis; that is, the cell has lost one of its primitive functions. The disposition of the chromatin seems to reflect this specialization by subtraction. Together with this decrease of the reproductive function, the cell has undergone a specialization by addition. The ameboid activity, the marked phagocytic and presumed anti-bacterial properties of the amphophiles are examples of such specialization.

In short, the polymorphous nucleus is not a sign of degeneration of the individual cell; but the disposition of its chromatin, just as in the lymphocyte, is a sign of a lost function. The cell not reproducing does not need a nucleus prepared for mitosis, hence the nucleus undergoes a regressive change.

Conclusions.

In the peripheral blood of the rabbit five distinct types of leucocytes can be distinguished:

I. Lymphocyte. Nucleus circular, chromatin generally in masses murally arranged. Protoplasm non-granular and strongly basophilic. (7 to 9 m. in diameter.)

II. Large mononuclear. Nucleus oval or curved, vesicular. Protoplasm non-granular, faintly basophilic. (12 to 16 m. in diameter.)

III. Amphophile. Nucleus polymorphous, chromatin in masses murally arranged. Protoplasm granular; granules small, ovoid, oxyphilic, may have selective affinity for acid dyes under certain circumstances. (10 to 12 m. in diameter.)

IV. Eosinophile. Nucleus polymorphous, chromatin in masses murally arranged. Protoplasm granular; granules large, ovoid, oxyphilic. (12 to 14 m. in diameter.)

V. Mast cell. Nucleus polymorphous, poor in chromatin. Protoplasm granular; granules small, spherical, basophilic, metachromatic. (10 to 12 m. in diameter.)

REFERENCES.

1. Hirschfeld. Virch. Arch., Bd. 149, p. 22. (1897.)
2. Muir. Jour. Path. and Bact., Vol. 7, p. 161. (1901.)
3. Scott. Jour. Path. and Bact , Vol. 7, p. 131. (1901.)
4. Ehrlich and Lazarus. Die Anaemie, Nothnagel's Spec Path. u. Therap., Bd. 8, pt. 1. (1900.)
5. Councilman. Jour. Boston Soc. Med. Sci., Vol. 3, p. 1. (1899.)
6. Taylor. Contributions from the Wm. Pepper Lab. of Clin. Med., Phila., Penn. (1900.)
7. Kanthack and Hardy. Jour. Physiology, Vol. 17, p. 81. (1894.)
8. Durham. Jour. Path. and Bact., Vol. 4, p. 338. (1897.)
9. Jolly. Arch. de Med. Exp., T. 10, p. 546.
10. Schreiber and Neumann. Fest. fur Max Jaffe. (1901.)
11. Dominici. Cited by Jolly.
12. Arnold. Virch. Arch., Bd. 140, p. 411. (1895.)
13. Councilman, Mallory, and Pearce. Diphtheria, Boston, 1901, and Journal B. Soc. of Med. Sciences, Vol. v.
14. Pratt. Contributions to the Sci. Med. by pupils of Wm. Welch, Baltimore, 1900, p. 265.
15. Mallory. Jour. Exp. Med., Vol. 3, p. 611. (1898.)

EXPLANATION OF PLATE XIII.

FIG. 1. Red blood corpuscle for comparison of size.

FIG. 2. Lymphocyte. Adult form showing chromatin in masses murally arranged.

FIG. 3. Large mononuclear. Showing vesicular nucleus and "ground glass" protoplasm.

FIG. 4. Mast cell. Showing chromatin poverty of the nucleus.

FIG. 5. Eosinophile. Showing massing and mural arrangement of chromatin. Note size and shape of granule.

FIG. 6. Amphophile. Showing massing and mural arrangement of chromatin. Note size and shape of granule and compare with mast cell and eosinophile. (Figs. 4-5.)

All camera drawings from smears prepared by Scott's method, except Fig. 4, which was from a dried smear stained in Jenner's stain. (Zeiss 2 mm. obj., no. 6 comp. ocular.)

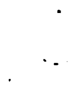


Fig. 1.

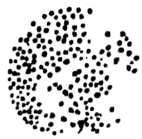


Fig. 4.

Fig 2.

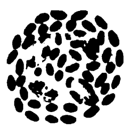


Fig. 5.

Fig. 3.

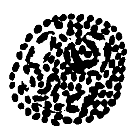


Fig. 6.

ON PHYSIOLOGICAL LEUCOCYTOSES OF THE RABBIT.

WALTER R. BRINCKERHOFF, M.D., AND E. E. TYZZER, A.M.

(From the Pathological Laboratory of the Boston City Hospital and the Sears Laboratory of the Harvard Medical School.)

It is known that in man and in many of the mammals in the state of health the leucocyte count varies within tolerably wide limits. Within these limits, placed approximately at 12,000 and 4,000 per cubic millimeter, there are daily and even hourly changes. On *a priori* grounds there is no reason to believe that the rabbit is an exception to this rule.

In a survey of the literature of experimental leucocytosis in the rabbit we have found the following observations which bear upon this subject:

Digestion Leucocytosis. — Rieder[1] (1893) details the following experiment, page 25:

"Ein Kaninchen, bei dem die Zählung unmittelbar vor dem Versuch die Zahl 7,000 ergeben hatte, erhielt am 20 Juli, Vormittags 10 Uhr, 180 cmm. ganz frisches, defibrinirtes Ochsenblut in den Magen vermittelst eines elastischen Katheters eingeführt. Nachmittags 3 Uhr hatte die Leukocytenzahl noch keine wesentliche Veränderung erfahren (sie betrug 7,200), ebensowening abends 6 Uhr; das Thier nochmals 200 cmm. desselben Blutes eingeführt, Tags darauf früh Morgens, den 21 Juli wurde dasselbe todt aufgefunden.

"Obduction: Der Magen war überfüllt mit Speisebrei, der Darm mit blutigem Kothe. Aus dem Rectum hatte sich eine grosse Menge schwarzen Kothes entleert. An den sonstigen Organen keine bemerkenswerthe Veränderung.

"Ob die Resorption von Hämoglobin oder eine andere Ursache den raschen Tod des Thieres bewirkt hatte, muss dahin gestellt bleiben.

"Obwohl al so die Eiweissstoffe hier in möglichst leicht

assimilirbarer Form dargereicht werden, trat dennoch keine Leukocytose auf."

We do not feel that this experiment is very conclusive. The food was not natural to the animal and too large an amount was given. Later in the same article Rieder says: " . . . ist beim Pflanzenfresser keine Verdauungsleukocytose nachweisbar."

Goldscheider and Jacob[2] (1894) make the following statement in the introduction to their admirable work upon experimental leukocytosis. (Kaninchen) "bieten den Vorteil . . . das die Zahl der weissen Blutkörperchen bei ihnen periodischen Schwankungen nicht unterworfen ist." They cite Pohl, Schultz, and Jacob.

Schultz[3] (1893) in studying digestion leucocytosis used rabbits as representatives of the class Herbivora. He details two experiments on rabbits, and in his conclusions makes the following statements:

"Beim Pflanzenfresser und beim jungen Fleischfresser, der wie jener fast stets in der Verdauung begriffen ist, werden *unter gewöhnlichen Verhältnissen* durch Nährungsaufnahme keine Veränderungen in der Zahl der im Blute kreisenden Leukocyten hervorgebracht. Wird jedoch bei ihnen künstlich durch längeres Fastenlassen ein Unterschied in den Verhältnissen vor und nach der Nährungszufuhr gesetzt, so tritt auch bei ihnen eine Verdauungsleukocytose auf."

The same author says: "Eine Verdauungsleukocytose existiert bei Kaninchen unter gewöhnlichen Verhältnissen nicht."

We are fully in accord with Schultz's statements, and the experiments to be detailed later are subject to the same interpretation.

Pohl[4] (1899) explains that he used dogs instead of rabbits for certain experiments upon chemical problems in digestion leucocytosis for "Kaninchen erwiesen sich zu den Versuchen ebenso ungeeignet, als wie zur Feststellung der Veränderungsleukocytose, weil es nicht gelingt, ihrer Magen-und Darm kanal durch Fasten ausreichend zu entleeren."

In these statements we find nothing to give support to the

view that rabbits will not show an ebb and flow in the number of the circulating leucocytes under conditions of intermittent feeding.

Other Leucocytoses. — Löwit[5] (1892) found that when a rabbit was tied down the leucocyte count decreased. He believed this phenomenon to be due to the restraint.

Goldscheider and Jacob (1894) showed that the decrease observed by Löwit was not due to the actual tying of the animal, but to the loss of body heat while stretched out on the "animal board." They show that by the use of a sand bath the body heat of the rabbit could be maintained and that then no decrease followed tying down.

Goldscheider and Jacob further showed that a "shock" would cause a decrease in the leucocyte count which was followed by a slight rise.

Recording Data. — The limits of accuracy of the Thoma-Zeiss apparatus for counting the number of leucocytes per cubic millimeter have been determined and we assume that, within one thousand cells per unit of volume, the leucocyte count is a true index of the corpuscular richness of the blood. A single count gives a true idea of the number of leucocytes circulating at the time of observation. It does not give any clue as to whether the number is rising or falling. In experiments on leucocytosis, what is wanted is information as to the change which is produced upon the number of circulating leucocytes. Obviously such information can only be had by comparing the successive counts, an interval of time elapsing between each. In a single animal where the leucocytes are counted at intervals and the changes are studied, the comparison of the various observations can be made directly. If, however, like experiments in different animals are to be compared it will be found convenient to express the changes which occur in each animal by a single figure. It has been shown that individual rabbits vary as to the number of circulating leucocytes. Consequently the absolute difference

between the counts in two animals cannot be fairly compared; for example, a decrease of two thousand in a rabbit having an initial count of twelve thousand with a like decrease in one with an initial count of four thousand.

We have found it convenient to express these changes in the leucocyte count as the decimal of a fraction whose denominator is the first count and whose numerator is the difference of the two counts to be compared. This figure represents the ratio of the change in the leucocyte count to the number found at the first of the observations compared. This "percentage change" will be prefixed with a minus sign when it is a hypoleucocytosis, with a plus sign when it is a hyperleucocytosis. The observations which are expressed with the percentage change will be shown by the letters of the observation placed before the numbers. Thus ac —20 per cent means that between observations a and c the count decreased twenty per cent.*

We have observations upon the following conditions, all of which we find affect the leucocyte count of the rabbit:

(1.) Fasting.
(2.) Feeding.
(3.) Pregnancy.
(4.) Certain affections to which the animal is subject.

The first three of these can be properly considered as physiological. The fourth might be better classed with the variations due to shock and loss of body heat as shown by Goldscheider and Jacob.

PHYSIOLOGICAL LEUCOCYTOSIS.

(*1.*) *Fasting.* — The following observations were made upon fifteen rabbits, eleven of which were killed within a very short time after the experiment. At autopsy none of

* Such refinment of data is obviously unnecessary in the study of the gross changes in the leucocyte count produced by most bacterial injections. We are here concerned with less marked variations.

the females were found to be pregnant, and no inflammatory lesions were discovered.

The body weight was noted at each observation, but is not recorded here, as it simply shows a progressive decrease. The leucocyte counts were made at the beginning of the experiment (the rabbit having been recently fed) and after various periods of fasting. The following tables give the results of the leucocyte counts stated in leucocytes per cubic millimeter as well as in percentage change:

TABLE 1.

Effects of twelve hours' fasting upon the leucocyte count.

Rabbit.	Before.	After.	Percentage change.
22	6,900	5,600	—19
33	9,300	5,700	—38
34	13,400	7,900	—41
50	8,300	7,300	—12
Average,	9,475	6,625	—30

TABLE 2.

Effects of nineteen hours' fasting upon the leucocyte count.

Rabbit.	Before.	After.	Percentage change.
109	7,200	6,000	—17
113	12,700	9,400	—26
115	8,000	6,800	—15
118	10,100	7;800	—22
110	11,300	8,000	—29
Average,	9,860	7,600	—23

TABLE 3.

The effect of twenty-four hours' fasting upon the leucocyte count.

Rabbit.	Before.	After.	Percentage change.
11	8,500	7,400	—13
21	6,400	5,400	—16
22	6,900	4,600	—33

Rabbit.	Before.	After.	Percentage change.
22*	11,000	9,200	—16
26	8,400	7,400	—12
34	13,400	7,500	—44
45	12,600	11,000	—12
46	6,900	5,300	—23
50	8,300	7,200	—13
94	7,700	5,400	—30
Average,	9,010	7,040	—21

Summary. — Fasting for twelve hours caused a decrease in the leucocyte in every instance. The decrease averaged approximately one-third of the initial count. Fasting for nineteen hours caused a decrease in the leucocyte count, but proportionately it was less than in the twelve-hour period. Fasting for twenty-four hours also caused a decrease in the count which was proportionately about the same as for the nineteen-hour period.

From these results it seems fair to conclude that in twenty-four hours' fasting the leucocyte count decreases more rapidly during the first than during the succeeding twelve-hour period. If anything, the leucocyte count tends to rise slightly toward the end of the twenty-fours of fasting.

The following observations bear out these views:

	Rabbit 34.	Leucocyte count.	Percentage change.
(a.)	At beginning of exp.	13,400	
(b.)	After 12 hours' fast.	7,900	(ab) —41
(c.)	" 24 " "	7,500	(ac) —44
(d.)	" 36 " "	9,000	(ad) —33
(e.)	" 48 " "	10,500	(ae) —20

In this case we have a considerable decrease in the leucocyte count for twenty-four hours, which is followed by an apparent increase.

Two weeks' interval between experiments.

	Rabbit 94.	Leucocyte count.	Percentage change.
(a.)	At beginning of exp.	7,700	
(b.)	After 24 hours' fast.	5,400	(ab) —30
(c.)	" 48 " "	6,700	(ac) —13

As in rabbit 34 we find a decrease in the leucocyte count during the first twenty-four hours of fasting which is followed by an apparent increase during the succeeding twenty-four hours.

(2.) *Feeding.* — Rabbits that had fasted for twenty-four hours were given as much carrots and hay as they would eat. The leucocytes were counted two and six hours after the beginning of feeding. The results were as follows:

TABLE 4.

Effects of two hours' feeding upon the leucocyte count of a rabbit that had fasted twenty-four hours.

Rabbit.	Before.	After.	Percentage change.
21	5,400	8,000	+48
22	4,600	8,100	+76
22*	9,300	14,600	+57
26	7,400	8,600	+16
50	7,200	9,600	+33
Average,	6,780	9,780	+44

TABLE 5.

Effects upon leucocyte count of six hours' feeding after twenty-four hours' fasting.

Rabbit.	Before.	After.	Percentage change.
21	5,400	8,000	+48
22	4,600	7,500	+63
22	9,300	16,400	+76
26	7,400	10,200	+38
50 †	7,200	8,400	+16
Average,	6,780	10,100	+48

* Two weeks' interval between experiments.

† Fed for first two hours only.

Summary. — Feeding a fasting rabbit causes an increase in the leucocyte count both two and six hours after the beginning of feeding. The increase is greatest during the first hours of the feeding period.

(*3.*) *Pregnancy.* — We have frequently observed that in a pregnant rabbit the leucocyte count is not decreased during fasting. In one instance a rabbit was killed for examination because it did not react to fasting and was found in an early stage of pregnancy. (Embryos 1½ cm.)

The leucocyte count also varies with age. We have often noticed that young rabbits have a higher leucocyte count than full-grown adults. The count in such animals is also less affected by feeding and fasting.

Certain affections to which the rabbit is subject:

*Snuffles.** — This term is used by rabbit fanciers to designate an affection in which the animal has a nasal discharge and sneezes frequently. A tenacious muco-purulent material is often expelled from the nares in paroxysms of sneezing. The disease is highly contagious, but rarely fatal. Rabbits with this affection do not show a decrease in the leucocyte count during fasting. At autopsy we have found accumulations of muco-purulent material in one or both nasal cavities, the condition frequently extending into the ethmoid cells. In the bronchi there is often histological evidence of an acute inflammatory process. In well-marked cases there are small areas in the lungs which show exudation of leucocytes into the air cells.

---

* Since the above was written, Dr. Theobald Smith has called our attention to an article by V. A. Moore and F. L. Kilborne ("An Outbreak of Rabbit Septicæmia," etc., Am. Vet. Rev., Vol. XVII, p. 285) in which they give an account of this disease and show its relation to the bacillus of rabbit septicemia. Dr. B. L. Thompson, of the Boston City Hospital, has isolated the same organism from two cases of the disease which occurred among our animals. From his studies (not published) of the organism present it seems to be more attenuated than the strain which Moore and Kilborne isolated. Our only interest in the affection arises from the fact that it is endemic among the rabbits that are available for our work. As the disease renders the animal unfit for experiments in leucocytosis its early recognition has been to us a matter of some moment.

The following experiment shows the effect upon the blood count of an infection with "snuffles":

Rabbit 25.	Leucocyte count.	Percentage change.
(a.) At beginning of exp.	6,600	
(b.) After 24 hours' fast.	10,700	(ab)+ 62
(c.) " 2 " feeding	11,400	(ac)+ 73
(d.) " 6 " "	14,700	(ad)+1.21

Animal was killed and numerous small areas of an acute inflammatory process were found in the lung.

INFLAMMATION FOCI ABOUT THE CECUM.

In two rabbits whose leucocyte count did not react normally during fasting, at autopsy the cecum was found studded with small circular opaque areas (2 mm.). In section these areas showed an acute inflammatory process.

Summary. — We find that during a short period of fasting fifteen rabbits show a decrease in the number of leucocytes. Eleven of these rabbits were killed and found to be without acute inflammatory processes. In those rabbits whose leucocyte count increased during fasting we found at autopsy either pregnancy or some inflammatory process of an acute nature.

If we are to draw inferences from the changes which take place in the leucocyte count during an experiment, it is important to know whether the count is going to change without our intervention. In other words, if we are to plot a curve we must have a base line. The following factors must be considered in drawing inferences from variations in the leucocyte count of a rabbit:

(1.) Loss of body heat (Goldscheider and Jacob).
(2.) Shock (Goldscheider and Jacob).
(3.) Fasting and feeding.
(4.) Pregnancy.

(5.) Snuffles and other spontaneous diseases of the animal.

Differential counts were frequently made, but showed little of interest. The leucocytosis following feeding was of the mixed type, that accompanying snuffles amphophilic.

CONCLUSIONS.

(1.) The number of leucocytes per cubic millimeter in the peripheral blood of the rabbit is constantly changing.

(2.) For the purpose of an experiment on leucocytosis we consider that only those rabbits are suitable whose leucocyte count decreases during a short period of fasting.

(3.) If the experiment is of short duration the animal may be kept without food. If of longer duration the time of feeding should be noted.

(4.) We consider that for a short experiment it is best to allow the animal to fast for twelve to twenty hours. If during this time the leucocyte count does not rise, the experiment may be carried out with the assurance that for a time the variations in the leucocyte count will be unworthy of consideration.

(5.) If during the preliminary period of fasting the count shows an increase the animal should be discarded.

The objection might be raised that a normal rabbit is in a constant state of digestion hyperleucocytosis and so would show no variation. Under conditions of constant feeding this is true. It is a matter of common observation that a rabbit in the presence of food will eat at frequent intervals. One rabbit was observed to eat quite regularly for a short time every hour. We believe that this condition of continuous feeding cannot be maintained during an experiment. Rabbits kept under animal-room conditions are not "unter gewöhnlichen Verhältnissen." And so Schultz's conclusions concerning leucocytosis in a rabbit, wherein he minimizes the influence of feeding, are not to be accepted unqualified.

(6.) If digestion leucocytosis be an increase in the number

of leucocytes due to feeding, our observations run counter to the general statement that herbivorous animals do not show this phenomenon.

We wish to express our thanks to Dr. W. T. Councilman for his advice and assistance.

REFERENCES.

1. Rieder. Arbeiten Med. klin. inst. München, A. 8, p. 185.
2. Goldscheider and Jacob. Zeit. f. klin. Med., Bd. 25, p. 373.
3. Schultz. Deutsch. Arch. f. klin. Med., Bd. 51, p. 234.
4. Pohl. Arch. f. Exp. Pharm. u. Path., Bd. 25, p. 51.
5. Löwit. Stud. z. Phys. u. Path. d. Blute, Jena, 1892.

"HANGING BLOCK" PREPARATIONS FOR THE MICROSCOPIC OBSERVATION OF DEVELOPING BACTERIA.

HIBBERT WINSLOW HILL, M.D.
(Director; Boston Board of Health Bacteriological Laboratory.)

The microscopic observation of the growth and development of the individual bacterium has been much neglected of late years. Cultural studies are pursued to the almost entire exclusion of this older and more direct method of investigation, and bacterial morphology has become largely a matter of dried and stained preparations.

Descriptions of bacterial species almost never include any observations on the development of the individual. It is usual to assume that the processes recorded years ago by De Bary and others for certain species may be safely inferred true of all species, new or old, without further investigation.

The distinguishing of species chiefly by cultural tests means a physiological rather than a morphological basis of classification. Physiological classification has a distinct value, and is essential in the present undeveloped state of bacterial morphology, but bacteriologists often deplore this departure of the lines of their systematic work from those of the botanist. It must be remembered also that the physiology of the culture mass is only partially, indirectly, and by inference the physiology of the individual bacterium.

It may be contended that the position of morphology in bacterial classification can never correspond exactly with its position in botanical classification. Whether this be true or not, there can be no question of the value of using morphological methods in the solution of morphological questions, yet the morphological question of the presence or absence of bacterial spore formation is more often tested by a biological (heat) than a morphological method, — indeed the recommendations of the Bacteriological Committee of the

American Public Health Association distinctly reject as a "required test" the actual observation of spore development, on the ground of its difficulty and tediousness. Examples might easily be multiplied, for the field is large and almost untouched.

It is hardly too much to say that the exclusive study of bacterial morphology in dried and stained preparations parallels the exclusive study of museum specimens of the higher plants. No botanist could rest satisfied with the latter, and bacteriology cannot but be the gainer by a more detailed study of the life cycle of the individual than has as yet become common.

The writer, desiring to attack from this standpoint the questions concerning the morphology of the diphtheria bacillus in general and the relationships of Wesbrook's nineteen morphological types in particular, was met at the outset by a series of difficulties in the existing techniques of hanging-drop examination. After numerous experiments, reaching interruptedly over some months, a form of technique was devised which is believed to be new, simple, and efficient. Some of the results already obtained relating to branching in *B. diphtheriæ* have been recorded elsewhere (Journ. Med. Research, Jan., 1902). Here the technique itself is described.

This technique consists briefly in substituting for the ordinary "hanging drop" of liquid or jelly a cube of solidified agar, on the surface of which the bacteria are distributed. The inoculated surface of this cube is applied to the under surface of the coverslip, and for convenience is known then as the "hanging block." Oxygen probably reaches the bacteria by diffusion through the block or the seal — certainly aerobic bacteria like *B. diphtheriæ*, *B. typhosus*, etc., grow readily in such a preparation. For anaerobes, it is sufficient to expose the block to the action of pyrogallol as described beyond. This method is applicable, as the hanging liquid drop is not, to motile as well as to non-motile forms. It is better than either of the older methods on optical grounds, because the bacteria present in a given preparation cannot lie otherwise

than horizontally and in the same plane. They are all necessarily in optical contact with the coverslip and are free to grow (horizontally) without the restrictions imposed by surrounding them with jelly or the freedom to drift allowed if liquid be used. For motility, the liquid hanging drop is of course necessary, but there is probably no other feature of the living individual which cannot be studied in "hanging block" preparations to advantage.

The "Hanging Block" Preparation.

Pour melted nutrient agar into a Petri dish to the depth of about one-eighth to one-quarter inch. Cool this agar and cut from it a block about one-quarter inch to one-third inch square and of the thickness of the agar layer in the dish. This block has a smooth upper and under surface. Place it, under surface down, on a slide and protect it from dust. Prepare an emulsion in sterile water of the organism to be examined if it has been grown on a solid medium or use a broth culture; spread the emulsion or broth upon the upper surface of the block as if making an ordinary coverslip preparation. Place the slide and block in a 37°C. incubator for five or ten minutes to dry slightly. Then lay a clean sterile coverslip on the inoculated surface of the block in close contact with it, usually avoiding air-bubbles.[1] Remove the slide from the lower surface of the block and invert the coverslip so that the agar block is uppermost. With a platinum loop run a drop or two of melted agar along each side of the agar block, to fill the angles between the sides of the block and the coverslip. This seal hardens at once, preventing slipping of the block. Place the preparation in the incubator again for five or ten minutes to dry the agar seal. Invert this preparation over a moist chamber and seal the coverslip in place with white wax or paraffin. Vaseline softens too readily at 37°C., allowing shifting of the coverslip. The preparation may then be examined at leis-

[1] Small scattered bubbles may at times be useful as further contributing to aerobic conditions.

ure. For *B. diphtheriæ* and organisms of similar size, Zeiss ocular 5, objective 1/12, oil immersion, and a Welsbach light prove satisfactory, although a lower ocular and higher objective are better. The Abbe condenser is not used. If preferred, the Welsbach light may be concentrated by a four-inch lens, focal length seven inches. An incandescent electric lamp is very difficult to focus and does not yield good results. Higher magnification than that provided for is of advantage, but requires more powerful artificial light.

Moist Chamber and Warm Stage.

Bacteria multiplying readily at room temperature can be observed in such a preparation exactly as an ordinary hanging drop is observed, except that the slide should be secured rigidly in some way to the microscope stage to prevent shifting. For bacteria growing best at 37° C. a warm stage is required. Of these there are many forms. The writer devised the warm stages shown in Figures 1 and 2.

In use, the warm stage (Fig. 1) is attached to a mechanical stage (the writer uses that of Bausch & Lomb) by means of the dovetail arrangement shown at (A). The binding screws (B) secure the warm stage rigidly in place. Two agar preparations are inverted over each chamber and sealed in place with white wax, paraffin, etc.; or, if only one preparation over each chamber is used, they may be secured by the coverslip clamps figured at (C). For prolonged observation, any opening left at the top of the chamber is covered by strips of glass sealed in place with wax. Water, air, etc., can be introduced into the chambers through the pipes (D) if desired. The stage temperature is adjusted by the circulation through it of warm water as described below. If slow-growing forms are under examination and it is inconvenient to have the apparatus run at night, the warm stage may be removed and placed in a 37° C. incubator in the evening and returned to the microscope next morning, the fields previously under observation being readily found again by the help of the vernier readings.

If anaerobic conditions are desired, a small cup containing

pyrogallol is placed in the moist chamber and the moist chamber is made air-tight.

Figure 2 shows a very simple and cheap form of warm stage, also suitable for use with the circulation apparatus described. It permits the observation of but one "hanging block" at a time, but very good results may be obtained with it.

"Retreaters," or clinical thermometers which fail to set, can be obtained for very little from any maker of clinical thermometers and do excellently for warm stage thermometers.

Circulation Apparatus for Heating Stage.

Figure 3 indicates the writer's apparatus. The advantages of this apparatus lie chiefly in its simplicity and cheapness. Several modifications may easily be made. If a large flask or other reservoir is used, the Bunsen flame may be applied directly to the flask and a thermo-regulator may be inserted into either bath or flask. The apparatus in use here requires the maintenance of a temperature of 56° C. in the water bath in order to obtain a temperature of 37° C. in the warm stage. A regulator is not usually essential. The temperature changes are slow, and since the observer must be more or less constantly at the microscope, he can easily supervise the temperature from time to time, and except when examining very slow growing forms, the observations need not extend over more than four or five hours usually. Indeed, when multiplication is active, the microscopic fields often become obscured in about this time by the overcrowding descendants of the original forms.

Comments.

It will be seen from the foregoing descriptions of apparatus that the essentials of successful work are few. Of these, rigid fixation of the bacterium in the preparation, rigid fixation of the hanging preparation to the coverslip, rigid fixation of the coverslip to the warm stage, and rigid fixation of the warm stage to the microscope stage, stand out prominently. If the microscope stage be a mechanical one, with

vernier readings permitting accurate measured movements, hundreds of individual bacteria may be kept under observation during one sitting by drawing the individuals seen in various recorded fields and returning to these fields from time to time in regular sequence. If the warm stage be large enough, the same species on several blocks of agar of different composition or several different species on the same kind of agar can be mounted side by side on a "serial-section" cover-slip, and since many fields in each preparation may be watched, a single successful sitting may yield a very plentiful supply of data. With a micrometer eye-piece and cross-ruled paper for drawing, or a camera-lucida, records to scale are easily kept. The details of and the time occupied in simple enlargement, fission, branching, spore formation, etc., may be ascertained.

The effect of variations in the composition of the nutrient agar from which the block is cut furnishes a wide field for investigation. For such work, different substances may be added directly to ordinary nutrient agar; or the agar may be used simply for its mechanical advantages by taking a three per cent solution of thread agar and adding it directly, before it solidifies, to any solution the effect of which it is desired to test. In such cases it is obvious that equal parts of the agar solution and the test solution should be used, and that the strength of the test solution itself should be double that which it is intended the hanging-block preparation shall have. Liquid blood serum, sterilized by filtration, or Wassermann's nutrose-serum solution may be added to such a plain agar solution, the latter being melted, but not above 50° C. at the moment of mixing.

Variations in the solidifying material of the "hanging block" have been tried. The writer has found that with very thin films of agar, bacteria may sometimes be stained under the observer's eye, but the details of the technique of this operation are not complete, and the results as yet have been irregular and unsatisfactory.

As an example of the kind of results to be expected, the following recent findings may be quoted: *B. diphtheriæ* shows,

as is well known, a parallel arrangement of the individual bacilli. This parallelism is due in many instances to a hinge-like movement on each other of the daughter cells derived by ordinary fission, a movement resembling somewhat the shutting up of an open penknife. Of this movement the first portion usually occurs instantaneously, the daughter cells springing from their original position in line with each other to an angular position, as if the open penknife were suddenly half shut or less. Thereafter parallelism is achieved by slow approximation of the distal ends. One or both of the rods must therefore rotate more or less in a horizontal plane about a vertical axis.

A parallel arrangement has also been found to develop in hanging block preparation of *B. typhosus*. In this species the parallelism is achieved by a slipping aside of the proximal ends of the daughter cells after fission and subsequent growth of the latter with little or no necessary rotation about a vertical axis. No portion of the movement occurs suddenly.

In *B. diphtheriæ*, therefore, parallel rods lie with the originally proximal ends adjacent to each other, and so of course also their originally distal ends. In *B. typhosus*, however, the proximal end of each member of such a pair of rods is adjacent to the distal end of its companion. The writer, using a dry lens, has found these observations true also in the absence of the cover-slip, which, therefore, cannot be regarded as influencing the results.

The observations regarding *B. diphtheriæ* are readily confirmed from stained preparations, since in these as well as in the hanging block the parallel arrangement is common and the proximal ends of short rods recently derived by fission can be easily distinguished from the distal ends because the former are usually the larger.

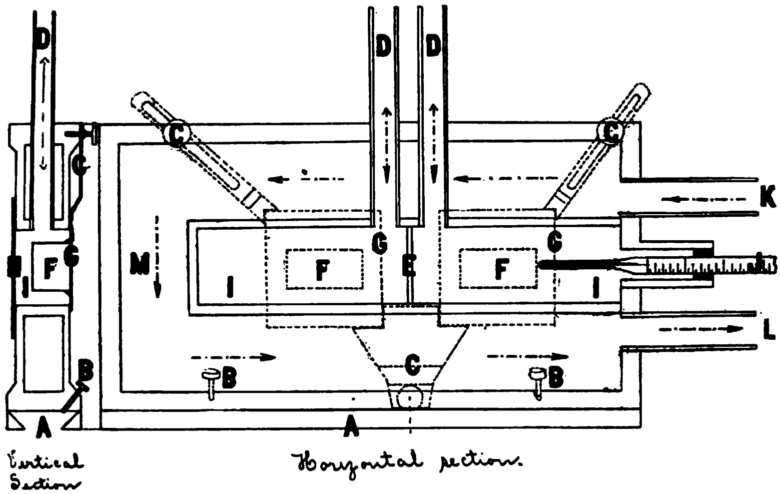


FIG. 1.

Warm stage; 3½ inches by 2 inches; made of brass.

A = dovetail arrangement for attaching warm stage to mechanical stage.
B = binding screws for fixing warm stage to mechanical stage.
C = clamps for coverslips, with set screws.
D = pipes connecting with moist chambers.
E = partition between chambers.
F = hanging agar block.
G = coverslip.
H = glass slide sealed to bottom of moist chamber with King's microscopic cement.
I = moist chambers.
J = thermometer.
K, L = inlet and outlet tubes for warm water.
M = water space.

Note. — The "vertical section" in this figure is not a true section, as a study of the horizontal section will show. It indicates together in one plane details which could not all be found in a true section, but serves the purpose of illustrating these details sufficiently well.

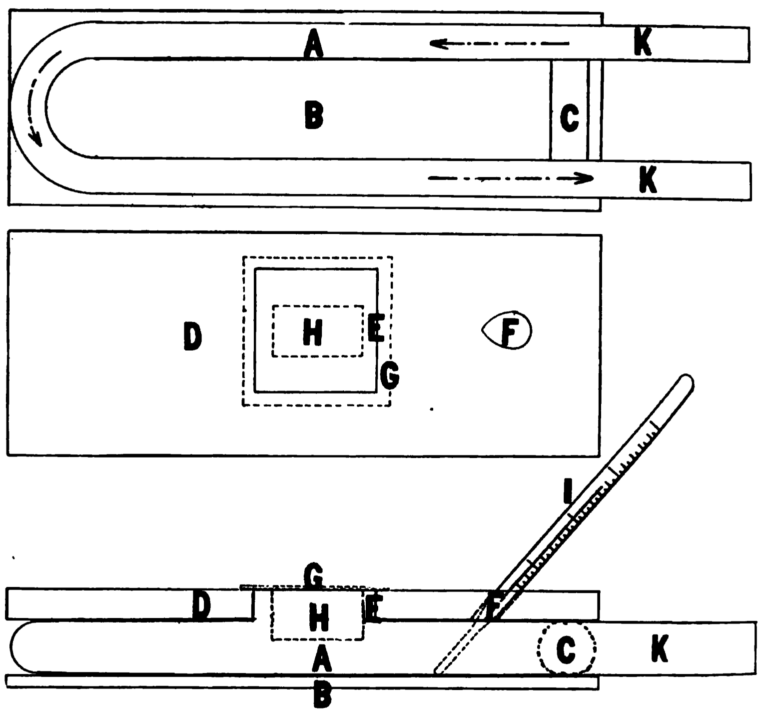


FIG. 2.

A = glass U-tube, outside diameter ¼ inch, distance between arms ½ inch, cemented to slide B.

B = ordinary microscopic glass slide (3 inches by 1 inch), cemented to lower side of U-tube A.

C = glass rod, ¼ inch diameter, cemented to arms of U-tube A, and below to slide B.

D = a piece of varnished or otherwise waterproofed wood, about 3 inches by 1½ inches by ¼ inch, perforated at E and F, and cemented to upper side of U-tube A.

E = square opening in wooden plate D for hanging block.

F = oval opening in wooden plate D for thermometer, adding water to the chamber formed by A, B, C, and D, etc.

G = coverslip.

H = hanging agar block.

I = thermometer.

K, K = inlet and outlet tubes for warm water.

King's microscopic cement, first well concentrated over a water or sand bath and applied hot, has been used with success.

A coverslip with a hanging block is shown in position by dotted lines. Its edges are sealed to D with white wax. The inlet and return tubes of the circulation apparatus are attached to the ends of the U-tube, which then forms part of the circulation system.

The projecting edge of the wooden plate D, purposely made thick, may be sealed to a mechanical stage with white wax. If a rotating stage is used, B may be sealed directly to the stage. If a plain stage is used, so that mechanical accuracy in movement cannot be achieved, it is best after finding a suitable field to fix the warm stage with the ordinary microscopic stage clips or seal it to the stage with wax.

Note. — The lower drawing in this figure is not a true section, but indicates the points it is desired to bring out.

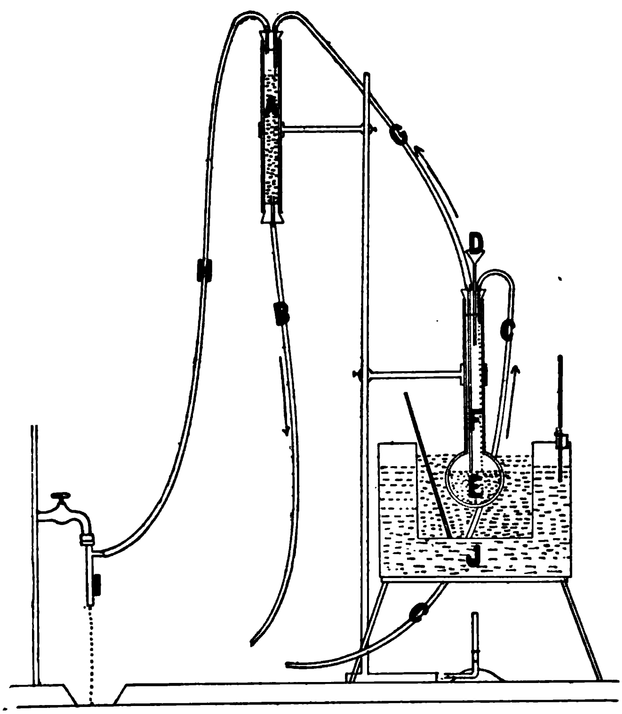


FIG. 3.

A = suction chamber, consisting of a glass tube about 9 inches by 1 inch, having perforated rubber stoppers with glass tubing leading to aspirator I, flask E, and warm stage.

B = rubber tube leading from chamber A to inlet of warm stage.

C = rubber tube leading from outlet of warm stage to flask E through rubber stopper.

D = small funnel for admitting air and also for replenishing system with water at long intervals, opening through rubber stopper into neck of flask E.

E = flask, of any size or shape, with rubber stopper perforated for inlet tube C, outlet tube F, and funnel D.

F = long glass tube passing from surface of water in bulb of flask E through rubber stopper to the rubber tube G.

G = rubber tube connecting flask E with chamber A.

H = rubber tube connecting chamber A with aspirator I.

I = aspirator.

J = water bath, with regulator if desired, for maintaining flask at same temperature.

With the aspirator flowing gently, a continuous but remittent circulation of the water is set up: from flask E to chamber A, by suction; from chamber A to the warm stage and back to flask E, by gravity. Water leaves the system only by evaporation and by the slight spray in chamber A, carried off by the aspiration.

The essentials of the apparatus (suction chamber and flask) may be set up in any way, perhaps most conveniently on an iron ring-stand. The relative vertical positions of these two portions must, however, approximate those shown.

Note. — As first designed, this apparatus had an additional tube between A and B, about the size of A, and used as a valve chamber. This contained an ordinary Bunsen valve of rubber tubing, arranged to allow a downward but oppose an upward flow. At the suggestion of H. D. Pease, this was discarded as unnecessary. It is possible that certain physical conditions of arrangement of the apparatus, rate of flow of the aspirator, etc., may make its introduction advisable, but under the conditions indicated in the plate, the apparatus works better without it.

A NEW SPOROZOAN PARASITE OF ANOPHELES.

HERBERT P. JOHNSON, PH.D.

(From the Laboratories of Comparative Pathology, of Harvard Medical School, and of the Massachusetts State Board of Health.)

In course of an investigation of mosquitos in a region where tertian malaria is endemic, undertaken under the direction of Dr. Theobald Smith for the Massachusetts Board of Health, about eight per cent of the females of *Anopheles maculipennis* were found to be infected with a sporozoan parasite presumably belonging to the order Gregarinida. Since no reference to the occurrence of this parasite in any of the Culicidæ can be found, and more especially since it bears sufficient resemblance to the oocyst or late amphiont stage of the malarial organism to render it liable to be mistaken for the latter (as in fact happened at the time of its discovery) the following brief and necessarily incomplete account is offered.

So far as present information goes, this little gregarine is found only in *Anopheles maculipennis*, which is well-known to be a species capable of becoming infected with malaria. During the investigation, which lasted from October 7 to November 5, 1901, three hundred and fifty-eight female mosquitos from the dormitories and hospital of the Boston Parental School, West Roxbury, were microscopically examined to ascertain to what extent they were infected with tertian malaria (*Plasmodium vivax*, Grassi and Feletti). The number of each species examined, which is also a fair index of its representation in the buildings at that time, is as follows:

Anopheles maculipennis	248
A. punctipennis	25
Culex pungens, C. pipiens, C. consobrinus	85
A. maculipennis infected with gregarines	20
Anopheles infected with malaria	0

While the number of *Anopheles punctipennis* is too small to warrant any conclusion as to whether it also harbors this parasite, it is evident that if it occurs in Culex at all it is less common in that genus. There is good reason to believe that the specimens of Culex examined (nearly all of them either *pungens* or *pipiens*) were bred in the same ditches and pools as the specimens of Anopheles; hence, apparently, the parasite had the same opportunity to enter them as the Anopheles. We know, however, that many gregarines are not less particular as to their host than are other sporozoa.

The parasite was not found in male mosquitos, but the number of these examined was too small to give any weight to a negative result.

Though the gregarines vary extremely in size (12μ–80μ), as may be seen in Figure 1, they are unfortunately all in the same vegetative condition. Not a single sporulating example has been found. All are simple cells, enclosed in a thin, structureless membrane, and are without epimerits or appendages of any sort. While usually attached to the peritoneal surface of the "stomach" (hinder portion of midgut), where they occur in numbers varying from one or two to more than fifty (Fig. 1.), they are by no means confined to this organ. Very frequently they are on the anterior portion of the midgut or on the Malpighian tubules, less frequently on the hindgut, and one was even found on the central lobule of a salivary gland. Frequently their attachment to the organs above-mentioned appears to be of the slightest. In only a small proportion is there a pedicle or process of attachment, as shown in the lower example of Figure 3. The latter are usually piriform, but other examples are extremely varied in shape. The fundamental form seems to be spheroidal; from this they vary to reniform, semilunar, lenticular, pear-shaped, and even to constricted and irregularly lobulated forms. The nucleus is nearly spherical, and contains coarse chromatin-granules; it increases with the growth of the cell (7μ–20μ in diameter).

The cell-contents of the parasite are as uniform as possible, and preclude the possibility that all the rather numerous

examples observed have represented more than one phase of development. Very rarely there are two nuclei (Fig. 3). The cytoplasm is invariably packed with reserve material in the form of spherical and oval bodies 3μ in diameter. These stain rather strongly with hæmatate of ammonia, Delafield's hæmatoxylin, and very strongly with iron-alum hæmatoxylin. Their chemical nature has not been investigated.

There is a rather constant and curious relation between the parasites and the tracheæ or breathing-tubes. These, as is well-known, form delicate and elaborate ramifications on the surfaces of the internal organs. The first few instances of tracheæ passing over the surface or even through the substance of a gregarine were thought to be somewhat in the nature of an accident. Subsequent cases became so numerous as to suggest some relation between the parasite and the breathing-tube, possibly that of respiration.

The presence of a parasite of any sort in the body-cavity of an animal presents a problem as to how it got there. It is known that some gregarines (*e.g.*, *Diplocystis major et minor* Cuénot, of the house-cricket) reach the peritoneal cavity by entering (in the encysted condition) the digestive tract of the host with its food. The digestive juices dissolve the cyst and the young gregarines work their way through the wall of the stomach.[1]

Although somewhat extended search of serial sections both of infected and non-infected material has been made, and not a single occurrence of one of these gregarines or of any cell-parasite in the walls of the digestive tract has been noted, no assertion can be made that they do not enter in this way. Their attachment to the stomach and other digestive organs certainly suggests such a mode of entry.

The entrance of the parasite with the food of the adult mosquito seems very doubtful, inasmuch as gregarines are not known as blood-parasites, nor are they known to occur even in the tissues or internal cavities of any vertebrate. It

[1] L. Cuénot. Recherches sur l'Évolution et la Conjugaison des Grégarines. Arch. de Biologie, tome xvii, p. 599, Pl. 20, Figs. 36, 37, 1901.

may, however, be taken in with the food of the larva; and this seems the most probable mode of invasion.

Since the body-cavity of an insect is a completely closed space, the exit of this cœlomic parasite from its host offers another interesting problem. It is known that female mosquitos not infrequently die immediately after laying, and their bodies remain on the surface of the water, there disintegrating. The gregarine sporozoites would then be liberated. If they float their chances of being taken in with the food of a surface-feeder like the Anopheles larva must be excellent, while there are difficulties in the way of entrance by the breathing tube, for the germs must pass from water to air, with which these tubes are always filled.

While simple contacts of the thickly-clustered gregarines, as Fig. 1 shows, are inevitable, contacts of a more complete and intimate sort are not infrequent. This is the so-called "conjugation" so common in gregarines (Figs. 1 and 4). Conjugated pairs are seen not only among gregarines in clusters, where they almost necessarily touch one another, but perhaps quite as often where they are few and scattered, therefore suggesting that a mutual attraction has been exerted. There is no evidence of a fusion of nuclei or even of cell-bodies in any of these conjugations. A septum has always been found between the conjugates. The most recent investigations on the fecundative phenomena of gregarines — those of Siedlecki [1] and Cuénot [2] — indicate that the supposed "conjugation" is not conjugation in the proper sense, but merely accouplement preparatory to encystment and sporulation. It has been frequently observed in various species of gregarines. The actual conjugation (fecundation), according to Siedlecki and Cuénot, comes later, when the daughter-sporoblasts unite in pairs.

In the living condition, as seen in physiological salt solution, the gregarines are almost transparent, and so slightly refractile that they may be easily overlooked, unless they chance

[1] Michel Siedlecki. Ueber die geschlechtliche Vermehrung der Monocystis ascidiæ Lankester. Bull. internat. de l'Acad. des sciences de Cracovie (Dec., 1899), pp. 515-537, 2 pls., 1900.

[2] L. Cuénot. *Op. cit.*, 1901.

to project beyond the outline of the digestive tract. It is in this condition that they are liable to be interpreted as the oocyst stage of the malarial parasite. Just how close the resemblance is cannot of course be stated by one who has not seen the malarial parasite under the same conditions. Judging by some of the figures of the latter given by Grassi and by Ross (compare Figs. 1, 2, and 5), the similarity is close enough to be rather disquieting to one who undertakes to make rapid diagnoses of mosquitos with a view to ascertaining the extent to which they are infected with malaria. The crucial point in the diagnosis is the presence of the single large nucleus in the gregarine, and many nuclei in the oocyst stage of the malarial parasite. In its uninuclear condition the latter is still very small (having simply changed from the ookinet without much increase in size), and is completely imbedded in the wall of the stomach.[1] Unfortunately, so far as yet known, the nucleus of the gregarine is not brought clearly to view without rather elaborate technique. The specimen from which Figures 1, 3, and 4 were drawn was fixed in aceto-sublimate (two hours), washed in water, and hardened in ascending grades of alcohol, one of which was iodized to remove the last traces of corrosive sublimate. It was stained with very dilute Delafield's hematoxylin for fifteen minutes (a shorter time — five or six minutes — has been found advantageous, as it does away with decolorizing), decolorized with acidulated alcohol, dehydrated, clarified, and mounted in balsam. Such a lengthy procedure is clearly out of the question when hundreds of mosquitos are being examined, and time and labor must be economized in every way possible. I have experimented with aceto-methyl green as a combined fixer and stain, and with osmic acid vapor, in both cases clarifying with glycerine; but neither has yielded satisfactory results. The nuclei of the parasites have remained obscure or even invisible, owing to the staining of the granules, while the nuclei of the mosquito tissues were beautifully stained.

---

[1] B. Grassi. Ciclo evolutivo delle Semilune. Ann. d'Igiene Sper., Vol. IX., Fasc. 3, 1899. Pl. 6, Figs. 19 A and B.

The desideratum for this work is a powerful nuclear dye that does not at the same time stain the reserve-granules, does not require extraction with acidulated alcohol, and permits combination with a fairly good fixing agent. Unless such a technique is devised and employed, or the tedious method of balsam mounts is used, any reports regarding malarial infection of Anopheles in this region are clearly open to criticism and doubt.

As in all work on parasites, much reliance in determinations must be placed on book-figures, which may be always at hand for comparison with the specimen. Accurate reproductions of figures by Ross[1] and by Grassi[2] (Figs. 2 and 5), representing respectively the infection of the stomach of Culex by Proteosoma (bird-malaria) and that of Anopheles by *Laverania malariæ* (æstivo-autumnal fever) are placed beside a similar figure (Fig. 1) representing the stomach of Anopheles severely parasitized by gregarines. If the specimen from which Figure 1 was drawn had not been stained the likeness would be considerably more striking.

If these organisms which have been identified as gregarines prove to be such, they will properly fall into Labbé's[3] sub-order Acephalina, since they lack the special organ of attachment (epimerit) characteristic of the Cephalina, and are for the rest of very simple structure. The best-known examples of the Acephalina are the various species of *Monocystis* in the seminal vesicles of earthworms.[4]

The present seems to be the first recorded instance of their occurrence in Diptera.

In the sub-order Acephalina the classification depends mainly on the method of sporulation and on spore-charac-

[1] Ronald Ross. The Rôle of the Mosquito in the Evolution of the Malarial Parasite. The Lancet, Vol. II., p. 488, 1898.

[2] B. Grassi. Die Malaria. Pl. 3, Fig. 40, 1901.

[3] A. Labbé. Sporozoa, p. 37, 1899.

[4] By far the largest number of the Acephalina are parasites of the cœlom or of organs in direct connection therewith. They occur mostly in Worms, Echinoderms, and Ascidians; but a few are known in insects both larval and adult. Perhaps the best-studied forms are the two species of *Diplocystis* described by Cuénot. These inhabit the cœlom of the house-cricket, which infects itself by devouring the dead bodies of its companions.

FIG. 1.

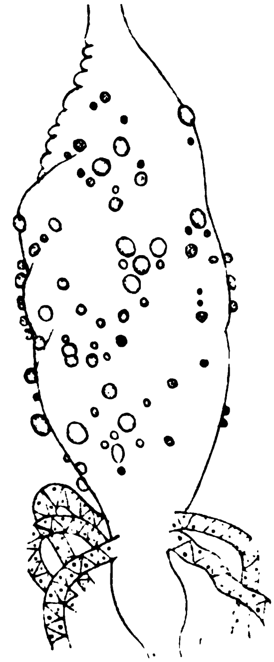


FIG. 2.

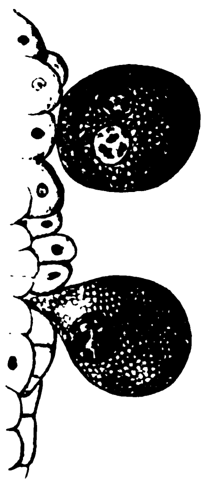


FIG. 3.

FIG. 4.

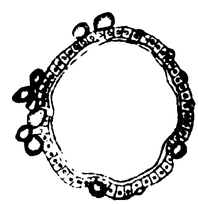


FIG. 5.

ters. Since these phases are yet unknown, the new form cannot be properly named, for it is impossible to place it in any particular genus.

EXPLANATION OF PLATE XIV.

(Figs. 1, 3, and 4 are from camera-lucida drawings by the writer. Fig. 2 is enlarged from Ross' figure as reproduced in Lühe's "Ergebnisse d. neueren Sporozoenforschung," Fig. 17.)

FIG. 1. — The midgut of *Anopheles maculipennis*, showing the severest infection found. Nearly all the gregarines are on the side towards the observer; those on the opposite side are not represented. The Malphigian tubules are omitted. (Aceto-sublimate, Delafield's hæmatoxylin, balsam.) × 65.

FIG. 2. — The enlarged posterior portion of the midgut of a Culex, beset with oocysts of Proteosoma (bird-malaria), 6–7 days after infection. After Ross.

FIG. 3. — Enlarged view of two gregarines also shown in Fig. 1 (upper right-hand side). × 500.

FIG. 4. — The large conjugated pair shown in Fig. 1. The larger of these represents about the maximum size observed. × 296.

FIG. 5. — Reproduction of a figure by Grassi (Die Malaria, 1901, Pl. 3, Fig. 40), representing a cross-section of the midgut of *Anopheles maculipennis* infected with advanced oocysts of *Laverania malariæ*, the germ of æstivo-autumnal fever.

STREPTOCOCCUS MUCOSUS (HOWARD) AND ITS RELATION TO MICROCOCCUS LANCEOLATUS.

WARFIELD T. LONGCOPE, *Resident Pathologist.*
(From the Ayer Clinical Laboratory, Pennsylvania Hospital, Philadelphia.)

Few micro-organisms are capable of retaining absolutely fixed biological and pathological properties under every condition to which they may be submitted. Growth under abnormal conditions and upon artificial media, exposure to high temperatures and chemical reagents; in fact, many circumstances both artificial and natural tend to produce slight changes in morphology, biology, and pathogenicity.

Micrococcus lanceolatus is not exempt from the general rule, and investigations made from time to time by different observers have established a somewhat wide range of variations for this organism. Among others Kruse and Pansini have made extensive studies upon the variations of pneumococcus.

As regards its morphology, the well-known lanceolate diplococcus is by far the commonest form, but a development of diplococci in chains is met with in a certain number of instances. The chains may be composed of lanceolate or flattened diplococci, the latter closely resembling streptococcus pyogenes (Kruse, Pansini, Welch, Bonome, Bordoni-Uffreduzzi).

Kruse and Pansini[1] found that long chains often reaching a hundred units were prone to develop after several generations on artificial media; and in old media involution forms were common. In rare instances the arrangement may be in typical biscuit-shaped diplococci.

Capsulation is a constant feature, but the ease with which the capsule can be stained is again variable. In animal exudates and pus, simple dyes are often sufficient for their demonstration; but in media it is often necessary to use milk cultures and stain by Welch's method. Ortmann[2] showed

that capsules could be stained in the first generation of blood serum, and associated the loss of virulence frequently found in the second growth with the loss of the staining power of the capsule. Kruse and Pansini came practically to the same conclusion and furthermore found that, as a rule, the capsule disappeared with the development of chain forms and the attenuation of virulence. In a few cases, however, the relations were reversed. Foá [3] isolated a variety from the sputum which showed stainable capsules in all solid media, and Welch [4] states that this may even be true of bouillon cultures. Gram's stain is invariable.

The biological properties may be very varied. Bordoni-Uffreduzzi, Kruse and Pansini, and others have noticed a luxuriance of growth on agar, single colonies often reaching two to three millimeters in diameter, and the latter observers have described a growth in acid bouillon. Fawitsky [5] has found that certain organisms produce a brownish pigment in fluid media. Growth in gelatine is not uncommon, according to Welch, being particularly noticeable in saprophytic varieties. Nikiforoff [6] has described a diplococcus identical with Micrococcus lanceolatus which does not acidify milk, and several observers have noted a faint visible growth on potatoes. On blood serum the growth is often luxuriant (Welch), but occasionally an organism, as that described by Bonome, produces no growth on this medium.

When the pathogenicity of the organism is considered, it is immediately apparent that the variation from a fixed type is even greater here than on artificial media. All grades of virulence and non-virulence are met with, even down to organisms which produce absolutely no effect whatsoever on animals. By prolonged artificial cultivation Kruse and Pansini were able to obtain these non-pathogenic varieties, and occasionally, even after two or three days' growth, the virulence is lost entirely. By a process analogous to this artificial cultivation, Fraenkel [7] explains the attenuated virulence of certain organisms isolated from chronic suppurating processes. On the other hand, Kruse and Pansini obtained virulent organisms from an empyema of six months' duration.

Foá and Bordoni-Uffreduzzi [8] believed that they could distinguish two types of pneumococci, a distinction based upon the lesions found in animals. While one form produces a general septicemia with an enlarged firm spleen and hyaline thrombi in the kidneys, the other is characterized by a more or less diffuse subcutaneous edema with small, soft spleen and bacteria rarely if ever in the blood. For a certain number of organisms this distinction appears to hold, but since Welch has shown that both forms of lesions may be produced by one and the same, Foá's theory cannot be accepted without reservation. Besides these two main types of lesions, many others may occur. Effusions into the serous cavities are common, and Welch states that if such effusion be old, they may present a glutinous consistence due to the great numbers of capsules. Biondi [9] describes a hemorrhagic edema about the point of inoculation, and Pasquale [10] has observed localized gangrene in rabbits. Emmerich [11] believes that Foá's division into groups is not sufficient to include all forms, and suggests that the variations are much more numerous.

From this brief resumé of the variations of Micrococcus lanceolatus it is evident that slight differences from the fixed type are scarcely sufficient to warrant the classification of certain organisms as definite species, merely because they do not conform to all the properties of Pneumococcus. Foá [12] at first thought that one of his variations was confined to the lesions of cerebro-spinal meningitis, and therefore termed it Meningococcus; while the other he believed to be the direct cause of lobar pneumonia. Later he recovered both the edematous and fibrinous varieties from pneumonic lungs, and thereby established the identity of the two forms.

The confusion which existed between Micrococcus lanceolatus and the organism of sputum septicemia was not entirely done away with even by the work of Klein [13] and others. For Biondi [14] in 1887 described an organism practically identical with Pneumococcus which he found in the sputum. This he called Bacillus salivarius septicus. In animals it produced a hemorrhagic edema at the point of

inoculation. Capsules were demonstrated by staining in eosin as well as by other methods. Kruse and Pansini do not hesitate to consider this bacillus the same as that of sputum septicemia.

Moreover, Pasquale[15] isolated what he termed "Streptococcus dell mucose" from the sputum of influenza patients. This differed from the typical pneümococcus inasmuch as it grew in large, transparent, raised colonies on agar, and produced a gangrene in rabbits after inoculation. There was no growth on gelatine, and in all other respects the bacterium conformed strictly to the growth of Micrococcus lanceolatus.

Binaghi[16] found a capsulated streptococcus in spontaneous peribronchitis and multiple pulmonary abscesses in a guinea-pig. The principle features were a marked capsulation, growth in long chains, and a rapid loss of virulence. The organism did not grow in gelatine. It stained by Gram's method. No essential differences exist between it and certain forms of Micrococcus lanceolatus.

From six cases of cerebro-spinal meningitis occurring in Padua, Bonome[17] isolated from the lungs and meningeal exudate of every case a capsulated streptococcus which he considered the specific cause of the epidemic. It did not grow on blood serum, but otherwise presented much the same cultural characteristics as Micrococcus lanceolatus. In animals, besides generalized fibrinous exudates upon the serous surfaces, a hemorrhagic subcutaneous edema was produced. Virulence was quickly lost on culture media. Bordone-Uffreduzzi[18] refuses to accept Bonome's streptococcus as a distinct species, and classes it among the varieties of Micrococcus lanceolatus. Kruse and Pansini as well as Frosch and Krolle[19] also consider the organism as an undoubted variety of Micrococcus lanceolatus.

Quite recently Howard and Perkins[20] have isolated a capsulated streptococcus which they believed sufficiently distinctive to represent a new species; namely, Streptococcus mucosus. The organism was obtained from the heart's blood, spleen, peritoneum, tubo-ovarian abscess, bladder, and right kidney of a woman who died of peritonitis and

pyelonephritis following a chronic tubo-ovarian abscess. An arrangement in long chains and biscuit-shaped diplococci was characteristic, and while capsules were especially heavy in animal exudates, they were not usually stainable in cultures on artificial media. The growth was somewhat luxuriant on culture media, appearing in gelatine and in the moisture about potatoes. Pathogenicity was rapidly lost on these media, and the first animal was killed only after repeated inoculations. As a differential point between this organism and Micrococcus lanceolatus, Howard emphasizes the subcutaneous mucoid edema which he frequently obtained in animals. Taking his as a type, he proposes to distinguish a small group of cocci standing between Streptococcus pyogenes and Micrococcus lanceolatus. Although most observers hold Bonome's streptococcus as a variety of pneumococcus, Howard included both this organism and Binaghi's Streptococcus capsulatus in the Streptococcus mucosus group.

The following bacteria which were isolated at the Ayer Clinical Laboratory during the routine bacteriological examinations bear a striking resemblance to Streptococcus mucosus. Inasmuch as one of them was found in the blood and consolidated lung of a man who died of acute lobar pneumonia, it is not impossible that they may help to bind the Streptococcus mucosus group even closer to Micrococcus lanceolatus than Howard considered it.

Case I.—J. M. Admitted to the Pennsylvania Hospital, August 31, 1901, with a swelling over the right chest. On incision a large amount of pus was evacuated. Some time after the operation, severe diarrhea developed, and the patient died September 30, 1901.

Autopsy revealed the following conditions: Subacute fibrinous pericarditis; abscess of the chest wall of left side; diphtheritic dysentery; compression of left lung with fibroid induration and bronchiectasis; amyloid degeneration of

liver, spleen, and kidney. From the pericardium and abscess was isolated a variety of Micrococcus lanceolatus with the characteristics to be described later. No other organisms were present in these situations.

Case II. — Ed. W. The patient entered the Pennsylvania Hospital October 14, 1901, and for five weeks suffered from a typical attack of typhoid fever. On the seventh day of normal temperature, pain developed in the left ear and two days later there was a slight purulent discharge from the ear. From the pus, Micrococcus lanceolatus was isolated, presenting the same features as in Case I. No other organisms were present either on coverslips or in cultures.

Case III. — Philip K. was admitted to the Pennsylvania Hospital November 14, 1901, with somewhat obscure symptoms and the history of an acute onset. On examination of the blood the following changes were found: Hemoglobin, 60 per cent; red corpuscles, 2,731,250; white corpuscles, 155,500.

Differential count:

Small mononuclear leucocytes	85.8%
Polymorphonuclear "	12.2%
Large mononuclear "	1.2%
Transitional forms	.4%
Eosinophiles	.4%

Diagnosis of acute lymphatic leukemia was made.

On the 19th of November signs of consolidation of the right upper lobe developed.

On November 21st, at one P.M., cultures were made from the median basilic vein. Death occurred the same day at nine P.M. Cultures from the vein gave a form of Micrococcus lanceolatus identical with that obtained from Cases I. and II. The organism developed on plates made of a mixture of agar and the patient's blood, in four bouillon flasks, and in one milk flask.

At autopsy the following conditions were found: Lymphatic leukemia; acute lobar pneumonia; acute fibrinous pleurisy; chronic interstitial nephritis; fatty degeneration of heart; splenic tumor. Cultures from the heart's blood showed numerous colonies of an organism which proved to be identical with that obtained from the blood during life. From the lung was isolated Staphylococcus pyogenes aureus together with enormous numbers of what at first appeared to be a typical pneumococcus, but which later developed the exact characteristics of the organisms from the blood.

The most important point in the last case is the distinct change which Micrococcus lanceolatus from the lung underwent after two or three generations on Löffler's blood serum. The first growth on various media adhered strictly to the common type of pneumococcus. Growth on agar was in small, transparent, gray, moist, dewdrop-like colonies; bouillon was not clouded, but a fine granular sediment developed in twenty-four hours. There was no growth either on potato or in gelatine. On the first blood-serum slant the growth was somewhat more luxuriant than on agar, but was quite meager in comparison to the subsequent growth on this medium. The organism occurred in lanceolate pairs, which rarely were connected to form chains. Capsules were only obtainable in milk cultures stained by Welch's method, and after treatment with iodine, according to the method of Gram, the organism retained the stain.

In sharp contrast to this somewhat delicate development was the subsequent growth of this organism, which corresponded exactly in morphology and biology to the coccus obtained from the blood in the same case as well as to those from Cases I. and II.

Although lanceolate diplococci were present, by far the commonest arrangement was in long chains composed of rather biscuit-shaped diplococci somewhat larger than Streptococcus pyogenes. The organism stained by Gram's method. But the most characteristic feature was the large size of the capsule surrounding both diplococcus and chain forms and the remarkable ease with which the capsule stained. While

this was especially true of the growth on blood serum, yet in all media large capsules could be demonstrated by staining one to two seconds in Gentian violet. Indeed, it was often necessary to decolorize the specimen for several seconds in 1–1000 acetic acid in order to differentiate the coccus from the heavy surrounding capsule. The capsules were absolutely constant in all media, and were quite as marked in transplants from the third blood serum growth of the organism obtained from the lung in Case III. as in the other cases.

The cultural characteristics likewise differed from the typical Micrococcus lanceolatus.

On agar plates the surface colonies often appeared as large, moist, glutinous colonies two to three millimeters in diameter. But this was not invariable, for occasionally the colonies were quite small and non-glutinous. Under the 3 objective they appeared finely granular and of a brownish tint. The deep colonies were oval or round, gray and translucent, and when magnified presented a definite granulation. Bouillon was not clouded and only after two to three days did a fine granular sediment develop. There was usually no growth on potato, but occasionally a faint, raised, moist line appeared along the streak. Milk was always acidified, but rarely clotted. In gelatine, after the first day, there was always a fine granular growth. On blood serum the growth was most luxuriant. Along the line of streak a raised, moist, pale, watery growth developed, which was distinctly viscid. The condensation water was markedly turbid, and in some instances became thickened and glutinous.

Inoculations into rabbits, guinea-pigs, and mice were made with the following results: rabbits proved quite insusceptible. They lived for two or three weeks after intravenous injections of one to two centimeters of twenty-four-hour bouillon cultures; but at autopsy cultures from the organs gave negative results. Guinea-pigs died from one to two weeks after large doses had been injected into the peritoneal cavity. At autopsy an organism presenting the exact features described above was obtained from the peritoneal cavity and liver. Mice, on the other hand, appeared more suscep-

tible. Death usually occurred one to three days following intraperitoneal inoculations, and an edema about the point of inoculation with sero-purulent effusion into the peritoneal cavity constituted the principal pathological findings. The spleen was occasionally enlarged and soft. In one or two cases the peritoneal exudate presented a glutinous character. An organism identical with the above was isolated from the peritoneal cavity and heart's blood of every case. Throughout these few animal experiments the bacteria retained all the peculiarities of morphology and biology observed in the original cultures, and the capsules, even in the last cultures, which were made over a month after isolation, showed the same marked affinity for aniline dyes.

It seems reasonably sure that the organism dealt with is a form of Micrococcus lanceolatus, although from a consideration of the first two cases alone this might not at first seem perfectly evident. From the fact, however, that in the last case a coccus first presenting the properties of the Micrococcus lanceolatus and later coinciding exactly with the capsulated streptococcus form was cultivated from a lung presenting the typical appearance of lobular pneumonia in the stage of gray hepatization, little doubt can be entertained that the differences in cultural characteristics and pathogenicity are probably dependent upon the environment and are not a fixed property.

As Kruse and Pansini have shown, when Micrococcus lanceolatus is cultivated through many generations on artificial media, the growth often becomes more luxuriant, chain forms develop, and the virulence is greatly reduced or entirely lost. Moreover, when the organism has finally attained this form it holds to it tenaciously, and only with great difficulty can one make it resume its original manner of growth. Since many observers explain in the same way the attenuation in virulence and altered biological characters of certain pneumococci isolated from chronic suppurating processes, it is not improbable that the apparently saprophytic type of

the present bacterium may be likewise accounted for in this way. Certainly in Case I. the process was at least of a month's standing, and the infection in Case II. ran an exceedingly mild course. Even in Case III. the mere fact that bacteria were obtained from the circulating blood before death does not necessarily imply a particularly severe infection, for Proschaka [21] has recently reported fifty cases of pneumonia in which he has cultivated Micrococcus lanceolatus from the circulating blood in every case. All the cases, he claims, were taken at random, and only twelve of the fifty died. Further, the pneumonia, although it was the immediate cause of death, occurred as a terminal event in an individual who must have been readily susceptible to an infection of low virulence.

Undoubtedly a close similarity exists between the present organisms and Streptococcus mucosus of Howard and Perkins. Indeed, the biological characters appear almost identical. Capsulation and low virulence are marked features of both bacteria, and the only tangible difference seems to be in the character of lesions in animals; and perhaps even these are differences more of grade than of pathological processes.

Under these circumstances the organism obtained from the pneumonic lung in Case III. may represent a transition stage between the typical Micrococcus lanceolatus and Streptococcus mucosus. In this event it would serve, perhaps, to bring Howard's group of capsulated streptococci under the varieties of Micrococcus lanceolatus.

In conclusion, then, it may be said that Micrococcus lanceolatus admits of a somewhat wide variation of growth from the common type; that these variations may occur as such in a certain number of pathological lesions; and finally that they cannot be considered as definite species, but should be looked upon as transient variations from the fixed type, their altered characters probably depending upon the peculiar conditions under which they developed.

REFERENCES.

1. Zeitschrift f. Hygiene u. Infektionskrank., xi, p. 279, 1891.
2. Arch. f. Exp. Pathol. u. Pharmacol., xxiv, p. 291, 1888.
3. Deut. Med. Wochenschrift, 1889, No. 2, p. 21.
4. Johns Hopkins Hosp. Bull., December, 1892, p. 125.
5. Deut. Arch. f. Klin. Med., l, p. 151, 1892.
6. Zeit. f. Hygiene u. Infektionskrank., xi, p. 279, 1891.
7. Deut. Med. Wochenschrift, No. 13, p. 209, 1886.
8. Deut. Med. Wochenschrift, xii, 1886.
9. Zeit. f. Hygiene u. Infektionskrank, ii, p. 194, 1887.
10. Giornale Internazionale delle Sc. Med., 1890, p. 602.
11. Zeit. f. Hygiene u. Infektionskrank., xvii, p. 167, 1894.
12. Zeit. f. Hygiene u. Infektionskrank., iv, p. 67, 1888.
13. Cent. f. Deutsch. Med. Wissenschft., xxii, No. 30, p. 529, 1884.
14. Loc. cit.
15. Loc. cit.
16. Centr. f. Bakt. u. Parasitenkunde, xxii, p. 273, 1897.
17. Ziegler's Beiträge, viii, p. 377, 1890.
18. Centr. f. Bakt. u. Parasit., vii, pp. 529, 670, 1890.
19. Flügge. Die Mikroorganismen.
20. Journal of Medical Research, vi, p. 169, 1901.
21. Deutsch. Arch. f. Klin. Med., lxx, p. 559, 1901.

A CASE OF THROMBOSIS OF THE CENTRAL VEIN OF THE RIGHT ADRENAL, WITH ENGORGEMENT AND NECROSIS (INFARCTION).

PAUL G. WOOLLEY, B.S., M.D.

Governors' Fellow in Pathology, McGill University, Montreal.

(From the J. H. R. Molson Laboratories of Pathology and Bacteriology.)

Apropos of the present case I have looked over the literature of the pathology of the adrenals without finding any that corresponds. Hematomata and blood cysts are reported by Carrington, Rontier, Cestan, Deroubaix, Letulle, Reclus, Fleischer and Penzoldt, Spencer, and Wainwright, and the literature is referred to and abstracted by Rolleston in his lectures on the Suprarenal Bodies. No case of infarction has been reported. The condition must be a rare one, for it is not mentioned by Welch, who does mention infarction following thrombosis of the mesenteric, the splenic, and the central retinal veins.

Hemorrhage into the adrenals is not at all uncommon. In one hundred and thirty cases of still-born children, Spencer found extravasation of blood in the cortex of the adrenals in two cases, and in the medulla in twenty-four cases; in three cases the hemorrhage had ruptured the capsule of the gland.

That such hemorrhage is not necessarily of immediate, serious import is illustrated by Wainwright's case in which the hemorrhage had become partially organized and absorbed. The present case illustrates the same thing in a different way and from the physiological point of view — for though the entire gland has been destroyed, the accessory glands remain apparently normal.

This case, for which I am indebted to Dr. Wyatt Johnston, was that of a female pauper child eleven months old who, previous to death, had had measles and was suspected at the time of death to have some throat trouble, and diphtheria had been suggested. This last could not be corroborated at autopsy.

When the abdominal cavity was opened a firm mass was felt in the right renal region which presented to the eye the appearance of a hematoma. This mass was about the size of a large goose egg. On closer examination it was found that the right adrenal was embedded in this mass, but the organ itself showed no signs of rupture; the origin of the hemorrhage could not be decided. The kidney was outside of the hematoma.

The adrenal itself was slightly enlarged and firm, but very dark, almost black in appearance, as from hemorrhage. On section the lines of the cortex and medulla could be seen with difficulty, and the entire substance of the gland was of practically the same consistency and of the same dark color. In the medullary portion and corresponding to the site of the central vein was a large round whitish mass, in size about that of an ordinary match, which had all the appearances of a thrombus. This could be followed throughout the length of the organ.

The left adrenal showed some hemorrhagic spots both in the medulla and cortex, but beyond this was fairly normal, although slightly fatty.

On microscopical examination of the right organ, the proper tissue of the gland seemed to have been completely replaced by hemorrhage except for the presence of occasional columns of palely staining nuclei surrounded by granular protoplasm, the sole remaining evidence of gland substance.

The thrombus was a laminated fibrinous one, with very few leucocytes, and with very little evidence of cellular structure, and there were no bacteria present in it.

Just outside the cortex of the gland and situated in the capsule were two very small accessory adrenal glands, appearing almost normal on microscopical examination, and showing no evidences of hemorrhage or necrosis. In the medulla was one other small nodule, encapsulated and apparently normal. All these must either have had a blood supply of their own or have been nourished by a collateral circulation which had been insufficient for the large gland.

The kidneys were cloudy, but sufficiently useful to all appearances, and showed no evidences of hemorrhage.

The origin of the thrombus could not be made out; it was probably marantic in origin, judging from its structure. It had been laid down gradually, for it was a laminated platelet thrombus. If the child had been younger it might have been ascribed to the trauma of birth, which is said to be the most common cause of adrenal hemorrhages. According to the well-known description of infarction by Welch, the present case is one of "Hemorrhagic Infarction of the Adrenal." Welch says that "if the veins are obstructed sufficiently to render the outflow nil, or very small, and the arteries are open, the infarction is intense. In consequence of abundant anastomoses of the veins, this mode of production of an infarct is rare, but it may occur after thrombosis of the mesenteric, the splenic, and the central retinal veins."

LITERATURE.

1. Welch. Embolism and Thrombosis. Allbutt's System of Medicine, London, 1899.

2. Rolleston. Goulstonian Lectures on the Suprarenal Bodies. Brit. Med. Journ., 1895.

3. Carrington. Blood Cysts of the Suprarenals. Trans Path. Soc., London, 1885, xxxvi, p. 454.

4. Rolleston and Marks. Primary Malignant Disease of the Suprarenal Bodies. Amer. Journ. Med. Sci, 1898, cxvi, p. 383.

5. Morris. Surgical Diseases of the Kidney and Ureter. London, 1901, Vol. II., p. 15.

6. Rontier. Cited by Morris.

7. Deroubaix. Contribution à l'Étude de l'Hémorrhagie de Capsules Surrénales, Paris, 1884.

8. Letulle. Cited by Morris and Cestan.

9. Réclus. Cited by Morris and Cestan.

10. Cestan. Cited by Morris.

11. Warthin. Adenoma of both Adrenals in the New-Born. Arch. of Pediatrics, Nov., 1901.

THE

Journal of Medical Research.

(New Series, Vol. II.)

Vol. VII. APRIL, 1902. No. 3.

COCCIDIUM INFECTION OF THE RABBIT'S LIVER.

E. E. Tyzzer, A.M.

(*From the Sears Pathological Laboratory, Harvard Medical School.*)

This disease is of common occurrence among domestic rabbits. In some rabbitries the larger number of the animals are affected to a greater or less degree. The object of this investigation is to throw light on the nature of the Coccidium infection, especially as regards its relation to tumor formation.

In the year 1839 Hake,[1] in his monograph upon Carcinoma, gives the following description: "There is a disease of the liver in the rabbit which consists of carcinomatous enlargements of the ducts, but manifesting itself under the form of small abscesses. This is owing to a dilatation of the ducts which occurs at regular intervals. The pus from these abscesses consists of ovate corpuscles, several times larger than the globule of ordinary pus." Although ignorant of the true significance of these "corpuscles," which were Coccidia bodies, Hake was the first to observe and describe them.

In the years following, these "ovate corpuscles" were repeatedly described as the ova of a certain species of Distomum,* and also as special types of cells. Long after the true parasitic nature of these bodies became understood, they were called Psorosperms, a name which has been in common usage even up to the present day. To Leukhart[2] is due the credit of bestowing the term "Coccidium" to this group of the Sporozoa.

In studying the resistant form of the organism, Balbiani[3]

* The similarity of the Distomum ova, found in the livers of certain mammals, to the resting form of Coccidium oviforme is so marked that the confusion of the two seems not unnatural.

found it to consist of an ovoid envelope, the "Oöcyst," enclosing a spherical granular body, the "Oöcyte." The latter became subsequently divided into four nucleated masses, the "Spores," each of which was surrounded by a distinct membrane.* Furthermore, these four spores were each transformed into two "Falciform corpuscles," the "Sporozoites." Thus was demonstrated a mode of reproduction through which one organism gave rise to eight new individuals. Balbiani believed the sporozoite to be the form which is active in the production of new infection.

Rieck [5] found experimentally that the gastric juice disintegrated the membranes investing the spores, and set the sporozoites free. He observed that these latter bodies were actively motile, wriggling here and there. From these observations he inferred that the sporozoites which were set free through the action of the digestive juices upon the spore membranes passed directly upward into the bile ducts, and thus infected the liver. It seemed unlikely that the sporozoites were carried to the liver by the blood stream, for the lesions were confined to the bile ducts in all cases.

The conditions necessary for reproduction to take place in the manner described by Balbiani are, that the coccidium first must pass in the feces from the body of the host, and that it must afterward be reingested. By this process each oöcyte gives rise to eight sporozoites. It is obvious that such a process of reproduction is inadequate to explain the severity and extent of the infection. The parasites are never evenly distributed throughout the liver, but are confined to certain portions of the bile ducts where they are found in great numbers. In the study of the development of the parasite within the host, Pfeiffer [6] observed the division of certain of these organisms into falciform bodies without any intermediate process of sporulation such as Balbiani had described. The falciform bodies so formed have since been termed the "Merozoites" (Simond), in distinction from the sporozoites proper, which arise from the ingested, resistant spores.

* Steida [4] (1865) furnished an excellent description of the formation of the four spores, also of certain stages of the process of shell formation.

The most complete work upon Coccidium oviforme has been furnished by Simond,[7] who has placed the mode of reproduction described by Pfeiffer upon an experimental basis. A pregnant rabbit was selected in whose feces no coccidia could be found. This animal was placed in a clean compartment and furnished with food free from all contamination. Five young rabbits were born, and were allowed to suckle for eleven days, during which time their dejecta were repeatedly found to be free from coccidia in any form. Four were then fed regularly upon sterilized milk. The fifth was isolated and fed for three days on sterilized milk with which were mixed large numbers of coccidia. This animal's apartment was kept scrupulously clean. It was subsequently fed on sterile milk alone, as were the others. On the eighth day after the first ingestion of the contaminated milk this rabbit became sick and its feces were found to contain coccidia. The number of coccidia in the feces was estimated and found to exceed by far any number which could arise from multiplication by eight, as would result from reproduction by sporulation. The animal's condition grew rapidly worse: diarrhea and convulsions followed. It was killed while moribund. The intestine showed intense coccidium infection. The feces of the rest of the litter remained free from coccidia throughout the experiment, and on post-mortem examination no lesions were found either in liver or intestine. The rapid progress and the extent of this infection demonstrated, beyond a doubt, the inadequacy of sporulation as the sole method of reproduction. Simond also describes, with great accuracy, as the "pseudo-flagellated" form of Coccidium oviforme, a large non-nucleated variety of the organism which has since been termed the "Microgametocyte," or male organism.*

Although unable to demonstrate it as fact, he advanced the hypothesis that these pseudo-flagellated forms underwent conjugation with other cells before sporulation could

* In his study of Karyophagus salamandræ and Coccidium proprium, Simond observed forms apparently analogous to this. Attached by one end to these forms were small "vermicular" bodies which were called by him "Micromerozoites."

take place. Thus did he not only demonstrate the polymorphism of the organism, but also suggested as a probable law that a sexual process must precede sporulation in all species of coccidia.

It still remained for Schaudin[8] and Siedlecki[9] [10] to trace the stages of nuclear transformation, associated with maturation, fecundation, and sporulation in the coccidia. Still more recently Lühe[11] has furnished a most comprehensive article upon the coccidia, adopting and modifying somewhat the schemes of Schuberg[12] and Schaudin to demonstrate the life cycle. He has also shown the relation of the malaria parasite to the coccidia, with which he believes it should be classed. The lesion produced by coccidium oviforme has been described by various observers, quite recently by Pianese.[13]

My own work was as follows:

Autopsies were made on thirty rabbits. Sections of liver from other sources were also studied histologically. Of the thirty rabbits examined the livers of seven were found to be affected. In no instance was there a demonstrable lesion in the intestine, although I have obtained preparations of such from other sources.

THE LESION IN THE LIVER OF RABBITS PRODUCED BY COCCIDIUM OVIFORME.

In these cases the livers contained small white or yellowish nodules measuring from three to ten millimeters in length (Plate XV., Figs. 1 and 2). They were usually pear-shaped or oblong, the longer diameter being about twice the shorter. The liver substance could be readily scraped away from these nodules, and they were found on dissection to be connected with the bile-ducts. In some cases the nodules were distributed throughout the liver substance. In others they were confined to one lobe. They were often apparent at the surface of the liver as slightly depressed, sharply defined, white areas. They were firm in consistency and moderately tough. On incision a white, cream-like fluid exuded. Microscopically examined, this fluid was seen to consist of

coccidia, biliary epithelium, granular detritus, varying numbers of leucocytes, and in many instances large numbers of bacteria. Falciform bodies were numerous, measuring about nine microns in length. None were in active motion. There were also minute, actively motile forms possessing flagellæ or tails.*

In certain animals, nodules were found much denser than those just described. On incising some of these a small amount of greenish, cheesy material could be expressed from the cut surfaces. This was found to be made up of coccidia with dense greenish-yellow shells. Other nodules were hard and solid throughout. There were ramifications of dense white tissue extending from these into the liver substance. The surface of the liver was dimpled and furrowed in the neighborhood of these nodules. Some such depressions were scar-like in appearance and a considerable degree of cirrhosis was present.

Histologically the nodules are of the nature of thick-walled cysts, papilliform in character (Plate XVI., Fig. 1). The cavity of the cyst is lined with epithelium of the type found in the bile-ducts. Most of the epithelial cells contain parasites which vary in size and character. The papillæ are made up of inpushing projections of connective tissue clothed with epithelium (Plate XVI., Fig. 2). In some cases the number of the papillæ with hyperplastic epithelium is such as to nearly fill the cyst, giving an adenomatous appearance to the lesion. In other cases the interior space is large and there are no papilliform projections; of the lining epithelium only a remnant is left, while the surrounding connective tissue forms a dense fibrous capsule about the whole lesion, thus encysting the enclosed parasites.

The remote changes in the liver, which accompany the presence of coccidium nodules, are those of cirrhosis. Around each of the smaller ducts is a hyperplasia of the connective tissue. In some instances septa of dense fibrous tissue extend outward from the nodules into the liver sub-

* These will be described more fully further on under the life cycle of the organism. Wasielewski [14] has observed these elements.

stance. With this condition there are often present many newly formed bile ducts, the so-called "adenoma" of cirrhosis.

LIFE CYCLE OF COCCIDIUM OVIFORME.

This parasite is of common occurrence in the feces of domestic rabbits; indeed, so common as to be almost invariably present. Many of the animals, however, in which it occurs seem to be in no wise affected, and when observed for periods of months remain in good condition. Post-mortem examination of such animals reveals no lesion, either gross or histological, although the parasites may be numerous in the intestinal contents. Their presence here is due to the fact that food is extremely liable to contamination with feces, and further that these animals often devour their own droppings, notwithstanding that they have food in abundance.

Extra-cellular Development of Coccidium Oviforme.— Only the spore-bearing form of the parasite is found in the feces. This form consists of a spherical, coarsely granular mass, the oöcyte, enclosed in a thick, double contoured, chitinous shell, the oöcyst. The oöcyst is ovoid in shape and varies greatly in size, some measuring eighteen microns, others as much as thirty-five microns in length. The smaller forms are almost transparent; the larger are colored a greenish-yellow as though stained by bile.* Each oöcyst has an opening at its smaller end.

The development of the oöcyte continues when subjected to a moderate amount of heat and moisture. It divides into two granular bodies, which in turn also undergo division. Thus are formed four nucleated bodies, the *sporoblasts*. After a time the sporoblasts become elongated, ellipsoidal in shape, and are also enclosed in a delicate surrounding mem-

* That the smaller variety of oöcytes are perfect organisms is shown by the fact that they divide, forming spores. It seems probable that such variation in size is dependent upon conditions affecting the nutrition of the individual coccidium. There is as yet no conclusive evidence of the existence of two distinct species of coccidia in the rabbit, although it is a noteworthy fact that the infection is confined to the liver in some animals and to the intestine in others.

brane.* The four bodies so formed are called *spores* and their surrounding membranes *sporocysts*. After being taken into the stomach, each spore divides into two falciform bodies, the *sporozoites*. The digestive juices probably act on the spore membranes so that the sporozoites are set free. Infection takes place by the entrance of the sporozoites into epithelial cells, either of the intestine or of the bile ducts. The precise manner by which this infection takes place has never been actually demonstrated. The sporozoites probably pass directly from the intestine into the bile passages.

Intra-cellular Development of Coccidium Oviforme. — An epithelial cell may be invaded by one or by several of these sporozoites. Within the protoplasm of the epithelial cell they appear as minute bodies, usually rounded, but occasionally elongated, and from two to three microns in diameter (Plate XVIII., Figs. 1 and 2). Each organism contains a darkly stained central body surrounded by a clear space, which is sharply defined from the remainder of its protoplasm. The central granule is deeply stained. The clear space is practically unstained. Simond regards the latter structure as the nucleus and the central granule as the nucleolus. Similar conclusions have been reached regarding analogous structures in other species of coccidia.† In this article the terms "nucleus" and "nucleolus" will be applied to them, although, as will be subsequently seen, their behavior differs somewhat from that of the nucleus and nucleolus in a typical cell. The nucleus is surrounded by a thin layer of protoplasm. The parasite and the protoplasm which it has invaded do not appear to be in immediate contact one with the other. Each young organism is separated on all sides from the cell protoplasm by a well defined space. It is impossible to say whether this appearance represents the true relation of the organism to the cell protoplasm, or an artifact produced by shrinkage in fixation. In sections a small crescent may be seen lying in contact with the surface of many of the small organisms which have penetrated epithe-

* In addition to the four spores, I have found a structureless mass situated within the shell.

† Siedlecki terms the darkly stained body the "karyosome."

lial cells. This crescent appears to be homogeneous and stains a uniform red with eosin. It seems to be of the nature of a disk resting upon the surface of the organism, and apparently forms no integral part of its structure. The apparent absence of this disk from some small organisms may be explained by the plane of the section. The parasite grows at the expense of the cell protoplasm in which it has lodged, and finally undergoes division, forming the so-called "falciform bodies" without an intervening process of sporulation.

The details of the process are as follows:

Asexual Reproduction — "*Schizogonie.*" *

Occasionally organisms of a relatively small size are found in which the nucleus has divided, so that they present two nucleoli, each surrounded by a clear space. Whether mitosis occurs here or direct division, it is impossible to ascertain on account of the minuteness of the structures. It is apparently a process of direct division. There is, however, no distinct nuclear membrane. The next stage is represented by cells with four nuclei and four clear spaces (Plate XVIII., Fig. 4). These clear spaces in the protoplasm often appear elongated and the nucleoli lie on the side toward the periphery of the cell.† Thin disks of the eosin-stained substance are found only occasionally on the surface of organisms at this stage. The protoplasm of the parasite increases in amount, but its staining characteristics remain the same through all the successive stages. It is slightly amphophilic, but shows more marked affinity for basic dyes. A reticular structure can be made out. The chromatic substance becomes further divided, each portion being invariably situated within a clear space in the protoplasm, until the organism is largely made up of these structures (Plate XVIII., Fig. 5). They are closely packed at the surface of the organism and are less so in its interior. The protoplasm about each clear space becomes denser and tends to take on a spherical form,

* Syn. — Endogene sporulation, Bildung von Schwarmesporen, Cycle eimerien, Cycle asporule.

† It may be that this arrangement is indicative of the tendency of these structures to place themselves at the surface of the organism.

and to become a separate unit. This form of organism, made up of smaller units, is termed the *schizont* (Schaudin). Thus the schizont becomes divided into spheres each having a distinct outline and each containing a mass of nuclear material, which at times is in the form of a rounded mass and at times irregular. The spheres become elongated and are transformed into merozoites, which may invade other epithelial cells and so bring about "autoinfection." Thus through asexual reproduction each undifferentiated organism gives rise to a number of merozoites.

The Merozoites.

The extreme variability of the merozoites, both as to morphology and arrangement, makes it difficult to describe them. In sections of fixed tissue, some are arranged in rosettes of fifty or even more, while others occur in groups of two or three. Some contain many scattered chromatin granules; others have a single nucleus with a deeply stained nucleolus. It is probable that the following conditions factor in this tendency to polymorphism which is evident with the merozoites: (1) The nutrition of the individual organism may at first influence its growth and later its activity in dividing. (2) The function of asexual reproduction may be diminished in the parasite after a number of generations, so that the merozoites of late origin vary in number and characteristic from the merozoites of earlier generations. (3) Certain differences of morphology may be due to the presence of various developmental stages of the merozoites. The following two types of merozoites may be regarded as the extremes of variation:

Those of the first and most common type (Plate XVIII., Fig. 7) show a marked tendency to arrange themselves with their long axes radiating outward from a centrally situated body, the "Restkörper."* Thus they appear in section to be arranged in rosettes. They are spindle-shaped with tapering, rounded extremities, and they each contain a clear space

* The Restkörper, I believe, represents a late product of the organism's division. In some instances it is degenerated and without structure, in others it shows the characteristics of structure and outline exhibited by the merozoites.

about a darkly stained body which is generally situated nearer the extremity toward the centre of the rosette. Still nearer this extremity a minute dark granule is demonstrable. The protoplasm is dense and seems to be finely granular. In section from fifteen to sixty merozoites are found in one rosette.

Merozoites of the second type are less numerous (Pl. XVIII., Fig. 11). An epithelial cell may contain as many as ten of these, but usually only three or four. With this type a structureless restkörper is occasionally met with, but is not constantly present. They are considerably thicker than merozoites of the first type and usually lie parallel with the long axis of the epithelial cell. The nucleus occupies a position at the middle of these merozoites and its outline is well defined. It contains a rounded nucleolus. These merozoites taper gracefully toward the extremities, which are rounded and are often bent upon themselves. The protoplasm appears to possess a looser structure as compared with that of the first type, but is denser near the extremities.

Only merozoites of the first type were found in smears. They varied somewhat in outline. With many, one extremity was drawn out and ended in a small nodule which contained the pigment granule above mentioned (Plate XVIII., Fig. 6). The full significance of the divergence of form is not understood. The possibility of sexual differentiation appearing here has suggested itself, but the stages which immediately follow these merozoites show nothing to justify the existence of two distinct sexes of merozoites. Merozoites of the first type are so extremely numerous that it seems improbable that they represent a single sex. It seems plausible that, if an epithelial cell becomes degenerated from the invasion of its protoplasm by several merozoites, the organisms return again to the "sickle" form within the cell, as they are not sufficiently nourished to complete their development and are too immature for reproduction. This would account for the existence of the large merozoites of the second type. Single organisms occurring within epithelial cells are occasionally elongated in form and resemble merozoites.

The merozoites which result from asexual reproduction enter other epithelial cells of the host and so extend the infection.

Sexual Dimorphism.

Besides the series of forms concerned in the process of asexual reproduction there are other forms of the organism which may be grouped into two complete series. One of these represents the development of the male organism, or " Microgametocyte," the other the development of the female organism, or " Macrogamete." After a process of maturation and fertilization,* the macrogamete develops a shell and becomes a resistant organism. Whether nutrition is the factor which results in the differentiation of sex or whether a sexual process is necessary after a certain number of asexual generations, as is the case with the Infusoria, has not been determined.

Sexual dimorphism exists in a species of coccidia inhabiting the intestine of amphibians, where the environment furnished the parasite is practically constant. The malaria organism apparently undergoes such sexual differentiation in the human blood. The sexual process, however, only takes place with a changed environment, that is, in the alimentary tract of the mosquito.†

The Development of the Male Organism, the Microgametocyte.‡

Organisms of relatively small size are found in which the nucleus has disappeared. Division of the chromatic material has progressed so far that it appears as minute darkly stained granules scattered throughout the protoplasm. Such organisms become microgametocytes. The protoplasm stains with basic dyes and has a loose, feathery structure. In more mature forms the minute chromatic bodies form a part of definite structures. They are surrounded by clear masses of

* The process of maturation and fertilization are to be presumed from the analogy of Coccidium oviforme to other species of coccidia, in which these processes have been traced. The presence of differentiated sexual forms also justifies this conclusion.

† Ewing.[15]

‡ This was formerly termed the "Pseudo-flagellated" form.

protoplasm which later take on an elongated form and come to be arranged with considerable regularity at the surface of the microgametocyte. In a later stage the chromatic bodies become more elongated and appear pointed at both ends. The protoplasm surrounding each of these becomes more and more drawn out and these structures become the fertilizing elements, the "Microgametes." The microgametocyte attains a relatively enormous size, thirty to forty microns (Plate XVIII., Fig. 8). They have a molten appearance, the surface bulging here and furrowed there, while portions of the interior are lightly stained. In the mature state, the microgametes are arranged at the surface of the microgametocyte from which they project. Filaments of protoplasm extend from these into the interior of the microgametocyte. In fresh material examined in five per cent salt solution there appeared minute bodies in active vibration at the surface of this form.

The Microgametes.

The microgametes are best studied in stained smears. They consist of a headpiece and two flagellæ (Plate XVIII., Fig. 9). The headpiece is from three to four microns in length and quite slender. It ends anteriorly in a sharp point and immediately posterior to this is a swelling which is less strongly stained than the remainder of the headpiece. The tails arise on opposite sides near the anterior extremity and measure six microns and ten microns respectively. The shorter is thicker and less tapering than the other.

The Development of the Female Organism, the Macrogamete.

This begins as a small body situated in the protoplasm of an epithelial cell. It differs in no known respect from the early stages of the schizont and microgametocyte already described. Its nucleus is relatively large and contains the nucleolus, which takes both basic and acids stains intensely. Simond describes in this form another crescent-shaped, chromatic granule situated near the nucleolus which he designates as the "Satellite nucleolus." The appearance of this,

he asserts, ushers in the " Cycle Sporule," or the process of Sporulation, thus marking the beginning of sexual differentiation. After careful search for this structure through many preparations with which different techniques were employed, no such structure as he described could be found. As the organism attains a larger size, there appears about the nucleolus an irregular mass of substance which stains very faintly with eosin. This cannot be properly termed chromatic.*

The disk of homogeneous, eosin-stained substance, already described in connection with the asexual form, is constantly present. In organisms which have attained greater volume (8–10 μ. in diameter), granules of similarly staining substance are found within the protoplasm (Plate XVIII., Fig. 12). Before the appearance of these granules and while they are still few in number, the protoplasm of the organism is markedly basophilic. It may be said with certainty that such organisms, containing acid granules, are destined to become oöcytes after taking part in a sexual process. The appearance of the acid-staining granules in their protoplasm marks their sexual differentiation. These granules become larger and more numerous with the growth of the organism. The latter grows chiefly through increase in the amount of its protoplasm, the growth of the nucleus being relatively less. The acid granules at first are scattered throughout the protoplasm, the larger being situated at the periphery. They increase in size with the growth of the parasite.

Following the appearance of the acid granules, a mass of basic staining material, presumably chromatin, appears lying near to the nucleus. In larger cells several of these masses are to be seen and are situated most commonly at and near the periphery. They are sometimes rounded, sometimes irregular, and sometimes skein-like in appearance. It is probable that the phenomena which these present are those of nuclear change associated with the process of maturation and fecundation, although the successive stages of these processes are not at the present time demonstrable. The

* "Chromatic" is here employed as "being of the nature of chromatin, a nuclear substance."

acid granules at first scattered throughout the protoplasm become arranged in concentric rings and are situated further towards the periphery of the cell, leaving a clear space about the nucleus. They become more and more irregular in form and finally become confluent. The nuclear membrane becomes irregular and shrunken and finally disappears, but the nucleolus persists for some time after it. After the migration of the acid-staining granules toward the periphery, they become confluent and their substance often appears in the form of a basket-work structure, situated at a short distance from the periphery of the parasite (Plate XVIII., Fig. 14). The material forming this basket-work loses its selective affinity for acid dyes and stains intensely with either acid or basic dyes. The layer outside this structure becomes differentiated and appears more refractive than the remaining protoplasm. It is at first relatively thick but appears to become thinner and condensed, so that in later stages it is represented by a dense refractive envelope, the oöcyst.

In the fully developed oöcyst, two distinct layers are demonstrable. The outer is colored with some staining reagents, the inner is not. It seems not improbable that the acid-staining granules function in the manufacture of this structure. The enclosed protoplasm now undergoes contraction and is represented by a coarsely granular mass, the oöcyst. After the formation of the oöcyst the parasite is set free by the degeneration of the epithelial cell which has harbored it, so that it may now be passed out of the bile ducts and intestine and leave the body. The life cycle is thus fully completed. Preservation of the species is provided for by this form of the organism, which is capable of resisting injurious agents and of reinfecting another animal.

Much may be said concerning the processes represented by the above stages. Do the infecting organisms grow feebler through successive generations of merozoites until they are incapable of further division, when they become sexually differentiated, — or is it lack of nutrition which produces this result? In support of the latter is the fact that in chronic lesions walled off by connective tissue, where the

soil is poor for parasite growth, the sexual and resistant forms are found to the exclusion of the asexual forms, the schizonts. On the other hand it seems likely that, if the rapid process of asexual reproduction were of itself to continue without limit, it would oftener end in the death of the host and thus provide no means for the furtherance of the coccidium species. Inasmuch as sporulation is inadequate to account for the multiplication of the organisms, it seems evident that asexual reproduction is for the purpose of multiplication of the parasite within the body or autoinfection, while sporulation accomplishes the continuance of the species through new infection.

The life cycle of coccidium oviforme may be summarized as follows: An oöcyst after being ingested may set free eight actively motile sporozoites in the digestive tract. The sporozoite may invade biliary epithelium. After acquiring its full growth (schizont) within the epithelial cell it breaks up, forming falciform bodies again, the merozoites (Process—Schizogonie). The merozoites invade the neighboring epithelium, thus causing autoinfection. After a time sexual differentiation takes place. Some organisms become macrogametes and probably undergo a process of maturation. Others become microgametocytes, from which the microgametes are derived, which are capable of fertilizing the macrogametes. The macrogamete after fertilization develops a resistant envelope, becomes free, and is passed out of the body of the animal. It is now in a condition to resist unfavorable conditions, until again ingested, when new infection is brought about.

The parasites attack only epithelial cells. The young form inhabits the protoplasm of the cell, which becomes more and more distended as the parasite develops. The nucleus, at first slightly indented, later becomes crescent-shaped. The structure of the chromatin cannot be made out, and the nucleus is stained darkly. Thus at the termination of this process, the epithelial cell is reduced to a sac containing a parasite, having on one side a darkly stained crescent, representing the degenerated nucleus (Plate XVIII., Fig. 14).

Ruptured cells are found from which the parasites have been set free. Degeneration and destruction of epithelial cells thus follow their invasion by parasites. Numerous mitoses are seen in the epithelium and, where the infection is not overwhelming, proliferation is in evidence. The epithelium is markedly thickened and its cells are crowded. Accompanying the destruction of single cells, exudative phenomena are absent. With the destruction of small areas of epithelium, there is exudation of fibrin and leucocytes (Plate XVI., Fig. 2). The latter occurs, however, only occasionally, and is the exception rather than the rule. When bacteria are present, the exudative phenomena are increased. The surrounding connective tissue is rich in cells. There are large numbers of lymphoid and plasma cells. Epithelioid and lymphoid cells occur between the cells of the epithelium. The epithelium oftentimes lacks a definite basement membrane, and young connective tissue and epithelium is so mingled that the resulting relations are decidedly confusing. Large phagocytic giant cells occur in conjunction with collections of oöcysts, the latter acting as foreign bodies. In some lesions large numbers of eosinophile cells are found scattered through the connective tissue.

The formation of the papilliform projections is to be explained by the hyperplasia of the connective tissue, which pushes through the defects in the epithelial layer. The question arises as to whether the proliferation is primarily of the epithelium or of the connective tissue. As proliferation is never confined solely either to epithelium or to connective tissue, it is to be presumed that the process involves both tissues at the same time. The steps of the process may be summarized as follows:

Following the invasion of its protoplasm by a parasite, the epithelial cell undergoes gradual but inevitable degeneration and finally becomes destroyed. The death of the cell produces a defect in the epithelial lining of the bile-duct. With the destruction of several adjacent cells the injury is greater and exudation of fibrin and leucocytes may take place. On account of the defect in the epithelium, the underlying con-

nective tissue is stimulated and proliferates. Pushing through the break in the epithelial layer, it forms the papilliform projections before described. At the same time the epithelium proliferates in an attempt to repair the defect in its continuity. As the parasites multiply, many mature forms become free in the bile-ducts, where they cause irritation, acting as foreign bodies.*

Evidence of this irritation is seen in the thickening and hyperplasia of the epithelium of the small ducts and in the hyperplasia of the surrounding connective tissue. In some instances the biliary epithelium is desquamated and portions of it are passed down the ducts. The epithelial cells often present a peculiar change in their nuclei (Plate XVII., Fig. 1). The chromatin is condensed into several intensely staining masses which lie against the nuclear membrane. The nuclear material apart from the chromatin is unstained. The nucleus as a whole is abnormally large and appears hollow. Councilman[16] has described similar changes in the corneal corpuscles of the rabbit's cornea. In that instance this arrangement of the chromatin preceded the direct division of the cell and was regarded as a degenerative change.

The actual mechanism by which the dilatation of the bile duct is affected, in the production of the coccidium nodule, is not clear. The stoppage of the duct, either through stenosis or by being plugged with organisms, would account for the dilatation. It does not seem probable that hyperplasia without stenosis could account for the formation of the lesion. In the more advanced lesion the central space is occupied by parasites in the resistant stage. The epithelium is degenerated in such lesions or even destroyed. The connective tissue is increased and forms a dense fibrous capsule, walling off further ravages of the parasite. There may be cirrhosis of the liver substance adjacent to the lesion. Where a considerable degree of cirrhosis occurs, there is generally a new formation of structures resembling small bile ducts. It is

* There is no evidence that the parasites of themselves secrete any toxic substance, but their presence in the biliary epithelium renders it more liable to bacterial invasion. In some instances the effect of bacteria is to produce an abscess cavity, in which but few coccidia are to be found.

possible to trace every transition between these structures and columns of liver cells. This new formation of bile ducts is analogous to the adenoma of cirrhosis elsewhere.

Repair takes place through the walling off of the lesion by connective tissue. Great numbers of the adult parasites are disposed of by phagocytic cells. Giant cells occur in the vicinity of the lesion with partially destroyed shells in their protoplasm. In some rabbits the only trace of the lesion found is a dense mass of cicatricial tissue with a few partially destroyed parasites included within the phagocytic cells. No metastases occur. The infection is confined to certain portions of the bile ducts. The lesions in a given liver are about equal in size, indicating that they did not arise one from another, but that all arose simultaneously.

Summary.

1. Associated with certain lesions in the liver of the rabbit are found parasites, of varying form and character.

2. The various forms represent the life cycle of a definite species of sporozoon, Coccidium oviforme. The necessary stages of the life cycle are traceable.

3. In only one stage does the parasite resemble the cell-inclusion of cancer. Even this stage presents a definite and constant morphology.

4. The immediate effect of the parasite upon the host is to produce degeneration and destruction of the epithelial cells of the bile ducts. Secondary to this, the effects of irritation are seen in the proliferation of connective tissue and epithelium. The more remote changes are those of cirrhosis.

5. Repair is effected through the walling off of the process by connective tissue, by the destruction of the remaining parasites, and finally by cicatrization.

In conclusion it may be said that there is no well-founded analogy between the cell-inclusions of cancer and any one of the stages in the life history of coccidium oviforme.

The lesion is of the nature of a chronic inflammatory process. The tissues react to the irritation which the para-

sites cause by their presence in the bile ducts. With the removal of the irritation, repair takes place. Thus the whole is to be regarded as a physiological process, checking the inroads of the parasite. It has little in common with new growth. No metastases are formed, nor is it possible for them to be formed, as is evident from the nature of the lesion. The process is self-limited and repair follows the destruction of the parasites.

[In ending I wish to express my gratitude and thanks to Dr. Councilman for his suggestions and for his personal interest in this work.]

REFERENCES.

1. — Hake. A treatise on varicose capillaries, as constituting the structure of carcinoma of the hepatic ducts, with an account of a new form of pus globule. London, 1839.

2. — Leuckhart. Die Parasiten des Menschen. Bd. 1, p. 254. Leipzig, 1879.

3. — Balbiani, G. Les organismes unicellulaires: les protozoaires. J. de microg., 1884, Vol. 7, p. 69.

4. — Steida, L. Ueber die Psorospermien der Kaninchenleber und ihre Entwickelung. Virchow's Archiv., 1865, Bd. 32, p. 132.

5. — Rieck. Dtche. Zeitschr. f. Thiermed. u. vergl. Path., 1889–90, p. 68.

6. — Pfeiffer, R. Die Coccidienkrankheit der Kaninchen. Beiträge zur Protozoen forschung. Berlin, 1892, Taf. 12, p. 24.

7. — Simond. L'Evolution des Sporozoaires du genre Coccidium. Annals de l'Instit. Pasteur, 1897, Vol. 11, p. 54.

8. — Schaudin and Siedlecki. Beiträge zur Kenntnisse des Coccidien. Sitzber. des Zool. gesell., 1897.

9. — Siedlecki. Étude cytologique et cycle évolutif de la coccidie la seiche. Annals de l'Inst. Pasteur, 1898, Vol. 12, p. 799.

10. — Siedlecki. — Étude cytologique et cycle évolutif de Adelea Ovata. Annals de l'Inst. Pasteur, 1899, Vol. 13, p. 169.

11. — Lühe. Ergebnisse der neueren Sporozoenforschung. Centr. f. Bact., 1900, Bd. 27, p. 367.

12. — Schuberg. Ueber Coccidien des Mäusedarms. Sitz. Phys.-Med. Ges., Würzburg, 1892, p. 65.

13. — Pianese. Le fasi di sviluppo de coccidio oviforme e le lesioni istologiche che induce. Arch. Parasit. Paris, 1899, Vol. 2, p. 397.

14. — Wasielewski. Ueber geisseltragende Coccidienkeime. Centr. f. Bact., 1898, Bd. 24, p. 71.

15. — Ewing. Clinical Pathology of the Blood.

16. — Councilman. The character of the cellular exudation in acute keratitis of the rabbit. J. Bost. Soc. Med. Sc., 1898–9, Vol. 3, p. 99.

For further references concerning coccidia, consult Lühe's article (*loco cit.*).

EXPLANATION OF PLATES.

PLATE XV.

FIG. 1. — The liver of a rabbit with a moderate amount of coccidium infection. The left lateral lobe contains several whitish nodules, from which is seen fibrous tissue extending outward into the liver substance.

FIG. 2. — The under surface of a rabbit's liver dissected in order to show the nature of the nodules and their connection with the hepatic bile ducts.

PLATE XVI.

FIG. 1. — Section of a nodule, showing the proliferation of the surrounding connective tissue and the papillomatous character of the lesion. Two bridges have been formed through the union of opposing papillæ. The space within the lesion is filled with oöcysts.

FIG. 2. — Section of a nodule with exudation of fibrin and leucocytes at (*a*) and (*b*). The epithelium covering the papilliform projections is loaded with parasites.

PLATE XVII.

FIG. 1. — A portion of a bile duct with its epithelium partially desquamated. The nuclei of the epithelial cells are degenerated and present four or five chromatin masses in contact with the nuclear membrane. To the right are parasites and cellular detritus filling the lumen of the duct.

FIG. 2. — A phagocytic giant-cell with an ingested oöcyst at (*a*). The giant-cell lies beneath the degenerated epithelium, which may be seen to the right of the field.

PLATE XVIII.

FIG. 1. — Young intracellular organism somewhat elongated.

FIG. 2. — An epithelial cell containing two young organisms, undifferentiated in type. Against the surface of the larger organism is a disk of eosin-staining substance.

FIGS. 3–5. Stages in the development of the schizont.

FIG. 6. — Merozoites as seen in stained smears.

FIG. 7. — Merozoites arranged in a rosette about the restkörper.

FIG. 8. — Adult male organism, microgametocyte. The dark stained elongated granules represent the heads of the microgametes.

FIG. 9. — Microgametes, as seen in stained smears.

FIG. 10. — Cross section of a group of merozoites similar to those seen in Fig. 11.

FIG. 11. — Large form of merozoites enclosed within an epithelial cell.

FIGS. 12, 13. — Stages of macrogametes immediately preceding the formation of the oöcyst. The dark stained granules are acidophilic.

FIGS. 14, 15. Macrogametes in the process of shell formation. In Fig. 14 the nucleus of the epithelial cell is represented by a dark stained crescent.

These drawings were made with camera lucida, Zeiss comp. ocular No. 4, one-twelfth homogeneous immersion.

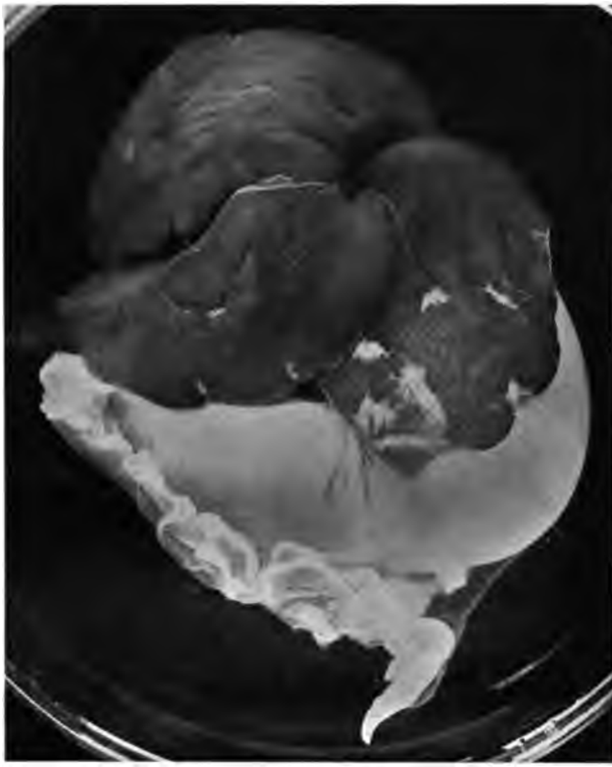


FIG. 1.

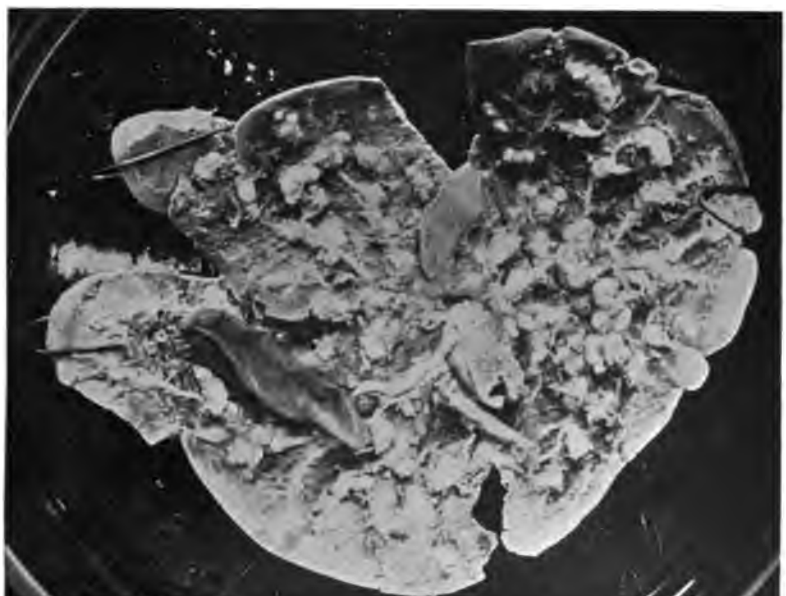
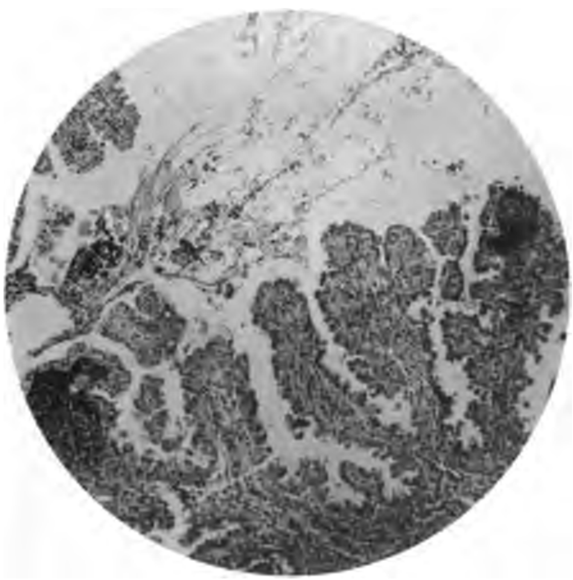


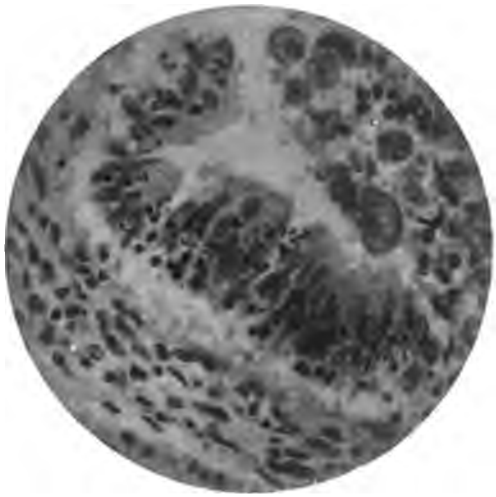
FIG. 2.

1



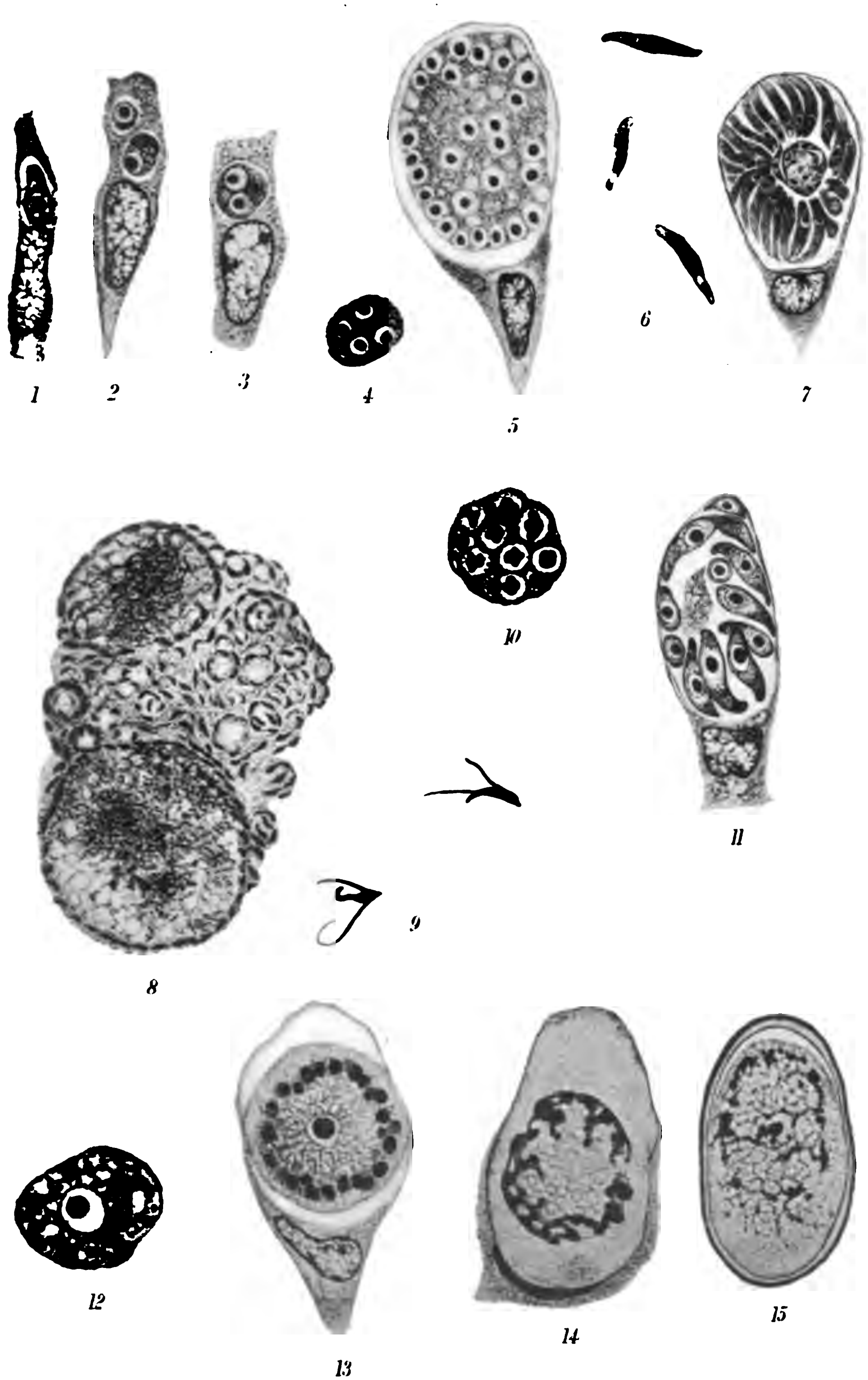
2

Heliotype Co. Boston



1

2



Tyzzer. Coccidium Infection.

MOLLUSCUM CONTAGIOSUM.

CHARLES J. WHITE, M.D.
(*Assistant in Dermatology in Harvard University*),
AND
WILLIAM H. ROBEY, JR., M.D.
(*Assistant in Bacteriology in Harvard University*).
(*From the Sears Pathological and the Bacteriological Laboratories of the Harvard Medical School.*)

The literature upon this subject is very extensive, for the peculiar histological picture presented under the microscope has been always a difficult one for pathologists to interpret and has given rise to many controversies among the leading investigators of the world. Therefore, before stating what has been observed in this present study of the disease, let me state, rather fully, perhaps, what has gone before, so that the reader may have a clear idea of the present status of our knowledge.

From the long list of writings enumerated at the conclusion of this paper I shall epitomize chronologically those which seem to throw individual light upon this much discussed subject.

The disease was first described in 1817 by Bateman,[1] who gave a very good clinical description of it and spoke of the peculiar matter, which appeared on pressure, as the secretion of the tumor. In 1814 Patterson[4] studied this "secretion" more closely and was the first to mention the strange, so-called molluscum corpuscles or bodies which appeared under the microscope. He described them as a peculiar sort of nucleus. Three years later Engel[7] regarded the tumor as an enlarged sebaceous gland, and in 1856 Rokitansky[10] and Hebra and contemporary English writers expressed similar views. In 1848 von Bärensprung[9] spoke of the molluscum bodies as epidermis cells which had imbibed albuminous matter and said that he had observed similar conditions in his investigation of sebaceous glands.

In 1865 appeared the result of Virchow's investigations.

Virchow [14] differed from the views of previous writers and claimed that the new growth was a lobulated glandular epithelioma, containing epithelial cells and a-nuclear bodies, arising from the hair follicles, and he described the bodies very carefully and likened their appearance to swollen starch bodies. He spoke of them as fat-like globules surrounded by double-contoured rims which resisted the action of water and acids, but became more translucent when subjected to the action of alcohol. He claimed that he had seen similar bodies in epidermoid cancer and "in the follicles of the nail bed" and said that they resembled the psorosperms found by Klebs [12] in intestinal epithelium; but nevertheless Virchow was a firm believer that he was observing a peculiar degeneration of epithelium.

Bizzozero and Manfredi [18] in 1871 published an article in which they confess their inability to state positively the nature and the development of the bodies, but believe that it is quite proper to affirm that they owe their origin to the protoplasm of the cells of the new growth. They noted that the bodies were insoluble in hot ether and in acetic acid.

This same year Retzius [21] denied the assertions of Virchow and claimed that the tumor did not arise from the hair follicles, but owed its origin entirely to the epidermis. This author further stated his belief that the bodies were *sui generis;* were never associated with cancer or epidermoid growths and did not arise from enlarged epidermic cells; and that their size only ("0.035–0.040 mm.") prevented him from considering them to be the spores of parasites.

Four years later Boeck [29] produced his careful paper which reads as though it were of much more recent date. He denies the idea of the sebaceous origin of the new growth because fat can never be detected in its cells. He found much resemblance in its structure to that of a gland and claimed that he detected evidences of vascular formations in its body. In the expressed material he described two forms of matter; first, the so-called molluscum bodies, which were round or oval, sharply bounded, transparent, non-nuclear, with a very doubtful double contour about them; and sec-

ond, strangely formed epidermic cells which were often without nuclei and possessed sharply bounded outlines. From this second type of cell Boeck asserted that the first arose, and claimed that he had seen the " bodies " within the walls of the peculiar epidermic cells.

Boeck's description, although now twenty-seven years old, is so excellent that it is a pleasure to quote from it at some length. "If one looks at the rete mucosum one will see that the first layers are normal, and then as one goes higher one will see that the cells soon become abnormal. If one looks at the space around the nucleus of these cells one will see a glistening rim. If one looks still farther one will see that the neighboring cells have become entirely composed of this rim. These cells are larger and universally rounder and one no longer sees the nucleus. This is the molluscum cell which one will see more highly developed higher in the rete. This gradual change is seldom seen, a fact which leads people to consider that molluscum corpuscles are foreign bodies. In the upper part of the rete one cannot find a nucleus. The difference in form of the cells (as one goes higher) is due to pressure, but the similarity of form (in lower part) speaks for the identity of the cells. The higher one goes in the rete the more prevalent become the bodies, until near the surface they constitute the whole mass. The protoplasm first changes and lastly the nucleus. Therefore, these bodies arise from a metamorphosis of the rete cell, not endogenously as Virchow claimed, but by a change in the protoplasm which first arises next to the nucleus. Chemical and physical tests show that these cells contain no fat. Chemical tests show that they are not amyloid."

That same year Lukomsky [30] stated his belief that the bodies arose from cells which had invaded the epidermis. The following year, 1876, O. Simon [32] made the statement that the tumor consisted of a colossal proliferation of the rete cells, and that the cell nuclei played no part in the production of the molluscum bodies. To his mind the gradual change seen in the cells and the great size of the bodies spoke against the theory of parasites put forward by Klebs [18]

and by Retzius,[19] but did not tend to prove that the bodies were produced from the protoplasm of the rete cells.

In 1877 appeared Kaposi's[33] views upon this subject. He was one of the first to agitate the question of the contagiousness of the disease and said that numerous fruitless attempts had been made by epidermal and sub-epidermal inoculations to reproduce the disease. Kaposi was a firm believer in the follicular origin of the tumor, but he protested against the idea that the molluscum bodies were parasites, for they never showed any signs of organic reproduction by proliferation or by budding; and he further disputed their origin from the cell nuclei, for he asserted that one could often see the nucleus squeezed up against the wall of the cell. He did believe that the peculiar bodies arose from the transformation of cell protoplasm, and said that this could be demonstrated by following up the changes under the microscope.

Vidal[39] in 1878 inferred from the jelly-like translucency of the bodies that they were the product of a colloid degeneration.

Two years later Renaut[50] stated his belief that it was in the sebaceous glands that the tumor originated, but confines the rest of his paper to the changes which occur in the rete cells. He then sketches the evolution of the bodies in the following manner: The protoplasm of the rete cells is composed of two substances; first, a hyaline material immediately surrounding the nucleus, and second, an outer zone. In the tumor the first layer of cells differs from the normal only in their larger size. Their perinuclear zone is large, translucid, and somewhat granular, and their cortical zone is thinner. In the higher layers this condition is still more observable and the cells become colossal with the nucleus pushed to one side, as a rule. In the meanwhile the cortical zone atrophies and becomes a thin envelope. This central "globulification" is not a fatty change, for osmic acid fails to color it. This change is not a death of the cell (and hence not colloid), for the cells take on later a peculiar cornification. At the level of the granular layer in the surrounding healthy skin we find a granular layer in the tumor containing kerato-hyalin in the

cortical zone of the cells and later a cornified layer as a cap. The tumor has connective tissue partitions. Renaut closes his article by stating that this process is a hyaline change occurring in the perinuclear zone of the rete cells.

The following year, 1881, Angelucci[55] described a bacterium — the bacterium lepogenum — which he claimed was the cause of the new growth, and in his review of this theory in 1882, Neisser[56] denied this and put forward his belief that the specific cause of the tumor was a gregarin and claimed that the peculiar bodies were the shells of these organisms.

Immediately afterwards Hebra[57] published his paper in which he ranked himself among the disbelievers in the contagious theory of the disease and among those who believed in the sebaceous origin of the new growth. At about the same time Caspary[59] produced a work in which he condemned the theory of the sebaceous origin by the fact that the smallest tumors never showed any opening upon the free surface, and also because no one ever saw such bodies in comedones or in atheromata. He believed that the tumor arose in the rete cells where in the first stages one could see the nucleus pressed to one side of the cell. He produced many good pictures which help the reader to follow his observations with clearness and satisfaction. He was a firm contagionist. Almost contemporaneously Geber's[60] excellent analysis appeared in which he sketched very accurately the evolution of the bodies from the rete cells. He said that the first change was a swelling of the palisade cells. Their protoplasm was distinctly granular, and their nuclei were distinctly enlarged, granular, and contained nucleoli, while around the nuclei a clear ring of protoplasm developed. Farther up in the tumor the cells became larger and irregular in shape, and the lighter perinuclear ring of protoplasm invaded the cell to a greater and greater extent until all else disappeared, leaving the molluscum bodies. In closing his article he said that molluscum contagiosum consisted in a hyaline degeneration of a hyperplastic growth of the interpapillary rete cells.

Allen's[64] paper, which appeared in 1886, dealt entirely

with the question of contagion. He related the importation of a case into an infant asylum in New York, and before a year had elapsed he was obliged to remove molluscum tumors from forty-two children.

In 1886 Neisser[68] again stated his firm belief in the parasitic theory of the disease and pronounced the peculiar bodies to be coccidia, a sub-class of the sporozoa. He described most minutely the various changes observed in the cells, but based his belief in the coccidial theory upon the fact that all the rete cells did not undergo these unusual metamorphoses. Neisser's description of the cell changes is so lucid that it should not be omitted here. The rete cell is at first enlarged and the prickles disappear. The nucleus, unaltered in size, shows often three to five long or round nucleoli. Later it grows smaller and alters its position in the cell and then is pressed to one side by the growth of a body in the cell — the parasite — and finally becomes crescentic. This nuclear remnant is always easily demonstrable even in the completed "body." The parasite, just mentioned, appears first near the nucleus and then grows larger. It is a fine granular mass with clear, transparent protoplasm and a marked cell membrane. In the later stages these bodies group themselves, but are separated by clear net-like matter.

In 1890 Török and Tommasoli[69] presented the most complete treatise which had yet appeared. They speak at first of contagion and relate several striking instances, placing the incubation stage at twenty days. They then relate their futile efforts at artificial cultivation on all known media and in the skin of guinea-pigs and in living cocks' combs. All experiments were hopelessly sterile. They then detail most minutely their significant chemical tests and demonstrate that the strongest acids and alkalies have but little or no effect upon the so-called parasitic bodies. They found that concentrated acetic acid swelled the body a little and rendered it more translucent; that strong sulphuric acid produced a similar effect; that strong formic and oxalic acid increased the translucency; that strong nitric simply changed the color; that concentrated hydrochloric acid cleared the bodies

and produced a slight granulation, and that strong hydrate of potash rendered them paler. They also learned that ammonia, ferrocyanide of potash, and permanganate of potash produced no changes, while digestion in sheep's gastric juice and in pepsin and hydrochloric acid simply rendered the bodies slightly granular. Certainly these demonstrations should eliminate once and for all any further belief in the coccidial nature of the molluscum bodies, and to strengthen still further the proof of their assertions Török and Tommasoli repeated all these experiments with known coccidia and produced entirely different results. They close their convincing arguments by saying that they believe these bodies to be the result of colloid degeneration.

Stanziale,[70] in 1890, asserts his conviction that the tumor arises in the rete cells, and states that these bodies react to caustic potash and to other chemicals not as parasites, but as a substance akin to horny matter.

Two years later Graham[75] adds his weight to the contagionists and says that he had observed a micrococcus in his cases which grew on potato and in bouillon, produced fermentation in milk, and was stained by Gram and by Gabbet's methods. Contemporaneously with Graham, McCallum[76] published his excellent paper. He limits the words " molluscum corpuscle " to the peculiar body inside of the epithelial cell, and according to him it first makes its appearance as a small spherule two micromillimeters in diameter in the third or fourth row of rete cells. At the same time a condensation of cytoplastic fibrillæ forms about the body which appears as a homogeneous, eosinophilous substance. The corpuscle then enlarges and the fibrillæ shrink, thus giving the cell the appearance of being surrounded by a doubly-contoured membrane. In later stages the cell stains homogeneously with eosin, hæmatoxylin, or safranin. Beyond the eleidin layer of epidermis the nucleus has disappeared and the cell stains bluish-violet with hæmatoxylin. Thus this body which at first was no larger than a nuclear plasmosoma (eosinophilous nucleolus) increases in size until it finally includes the contents of the nucleus and the major part of the

cytoplasm. McCallum regards the first change in the process as an extrusion of a plasmosoma.

This same year Pick[77] records himself as a believer in the parasitic nature of the bodies, and relates a successful inoculation upon a human subject.

The following year, 1893, brings us to the third publication of Neisser,[78] the chief exponent of the coccidial theory. He advocates the examination of the bodies in fresh frozen sections, and summarizes his views as follows: The bodies are parasitic because, first, no degenerating cells look like these bodies; second, analogous bodies can be observed in other sporozoa; third, these cells, which are without analogy in human pathology, are met with in a truly contagious and inoculable process; fourth, the conditions of this tumor are unique in epithelial new growths; fifth, we have to do with a new growth whose cells are in part only affected with degeneration, whose nucleus, although pushed to one side, remains always intact; and sixth, the epithelial growth is quite different from that occurring in carcinoma and all other epithelial hyperplasias — papilloma, verruca, etc.

Crocker,[80] in 1893, ranks himself among the believers in the follicular origin of the tumor. He places the statistical frequency of the disease at two in every thousand cases of skin disease, and says that the tumors can grow everywhere except on the palms or soles. He regards the fibrous septa seen in the tumor as remnants of papillæ.

In 1894 Hansemann[82] states that he never found a nucleus in the bodies, or a trace of any organization. The following year Stelwagon[83] elaborates the inoculation theory, and divides transference of the disease into four varieties: first, that in members of the same household; second, that among inmates of asylums, schools, and hospitals; third, that produced by accident; and fourth, that resulting from experimental inoculations. He notes the interesting fact that accidental inoculations result in tumors at the end of a week or more, while experimental tumors require nine to twelve weeks for their development. About this same time Kuznitzky[84] places himself on record as a contagionist, as a believer in the epidermal

origin of the tumor, and as a disbeliever in the parasitic nature of the bodies. He describes the "corpuscles" as homogeneously glassy, without the least granulation or sign of any nucleus. He states that alcohol is a very good hardening agent.

Touton [85] immediately replies to Kuznitzky's article decrying the value of alcohol as a preservative, and places himself by Neisser's side as an upholder of the parasitic theory. He maintains that these much discussed bodies must be organisms, because he knows of no known homogeneous degeneration(!).

Benda [86] also, in 1895, published a paper which records some of the most careful observations which we have upon this subject. He feels that in some cases the tumors do arise in the hair follicles. He notes that there is no inflammatory reaction about the tumor, and that there is no increased mitosis, and that whenever mitosis occurs it is limited to the palisade layer — an exception from other epidermal new growths. Benda believes that the increased size of the tumor is due to hypertrophy, and not to hyperplasia, and propounds the interesting theory that the cells become larger because normal keratinization is delayed by pressure from above — a condition which determines the growth of the tumor mass downwards and sideways.. He continues his interesting ideas by stating that the bodies are epidermal cells with abnormal contents, and outlines the successive steps in this strange metamorphosis as follows: In the lowest layer of the rete the only abnormality is that cells are pressed together, and intercellular spaces and bridges disappear. In the third and fourth layers a strange body is seen in some of the cells. These bodies are about twice as big as a nucleolus, round, elliptical, or sickle-shaped, and lie near the nucleus. There may be two of these bodies, in which case they do not lie so near to the nucleus. These bodies are always surrounded by a clear zone which is a vacuole, in all probability. Higher up only one body appears, and this presses the nucleus to one side, and still later this body is replaced by a fine network of fibres. The nucleus is now pressed more to one

side, and the whole cell is much increased in size. The nucleus retains its membrane and its nucleolus. Some vacuoles appear in the cells, and these grow in number and in size, and eventually fill up the whole cell, leaving only septa of the original network. These changes occur at the level of the granular layer, and we find here a double wall with nucleus in the outer ring. Neisser looked upon these divisions of cells as evidences of spore formation. When the stratum lucidum is reached these divisions begin to disappear, and in the stratum corneum the whole contents become homogeneous, and receive solidly basic anilin colors, and the cell as a whole shrinks in size.

Benda believes that the peculiar body is a parasite because, first, it takes a color so characteristic of known parasites, *i.e.*, anilin gentian violet; second, it is so sharply separated from cell protoplasm; and third, on account of the number and shapes and sizes of these bodies in one cell which can be interpreted only as segmentations of a parasite.

In 1896 appeared Unna's "Histopathology," in which the author gives a most thorough elucidation of this complex question. Unna[83] prefers the title of epithelioma contagiosum suggested previously by Virchow and by Neisser. He does not believe in the sebaceous origin of the tumor because he has never seen any sebaceous material whatever in the tumor tissue. He accounts for the rounded shape of the tumor by the fact that not only do the cells proliferate, but also because they swell enormously. He states his preference for the polychrome methylin blue glycerine ether method for the study of these protoplasmic changes and describes the metamorphosis in this manner: We see the precursors of the bodies in the lowest layers of the prickle cells which swell by the increase of their colorable protoplasm. At the distal end of the cell we note a deeply stained granular mass wherein the first pathological changes appear. The normal dark blue stain becomes slightly greenish, the dense granulation disappears, and the cell contents become more homogeneous. The nucleus lies in the opposite cell wall which is distended into a thickened capsule. The vacu-

oles which now appear are due to alcohol hardening and are of no consequence (!) As cornification advances it is probable that some protoplasm is converted into colloid matter. Cornification takes place in the outer portions of the cell while the colloid degeneration occurs within. The resulting bodies appear as light, unstained, oval or round structures surrounded by kerato-hyalin envelopes.

Thus we see that the "bodies" are only the results of colloid or hyalin degeneration of the prickle cells; and this differs from other hyaline degenerations (in carcinoma and in psorospermose folliculaire végétante) because it occurs in interior of cells only, while usually it is the ecto-fibrillation which undergoes this change. In most cases the neighboring structures show no abnormalities. At times vessels are dilated, connective tissue cells are multiplied, plasma and mast cells are increased, and numerous mitoses are noted, but in such instances cocci are present and we are dealing with a mixed infection.

Audry,[90] in 1899, produced the last important monograph upon this subject and he records himself as a disbeliever in the pilo-sebaceous seat of origin of the tumor. He states that the growth is surrounded by a connective tissue shell which never presents any signs of inflammation unless secondary changes are present. He describes the "metaplasia" as follows: In the cylindrical layer the cells are well preserved, but we find an increase in the number of mitoses. In the following layers we find a dilatation of Ranvier's perinuclear space. This is not produced by alcohol, for we observe the same anomaly in cells hardened in osmic acid. The nucleus ceases to be uniformly granular and clears up somewhat while its chromatin gathers at several points resembling nucleoli. The cells lose their prickles, develop a membrane, and grow larger, while their protoplasm becomes granular. The higher one goes the more marked these changes, and we find the mantle becoming more distinct and the protoplasm seeming to float within this envelope and at times escaping from it. The nucleus is displaced to one side, assuming an oval and later a falciform shape, and then we

note the formation of transparent and refractile vesicles. At the level of the stratum granulosum eleidin appears in the outer part of the cells and finally at the site of the horny layer we find normal kerato-hyalin. Audry states that the substance of the bodies is not homogeneous, but is composed of little blocks which, however, cannot be termed granular.

The most recent description of this disease comes from Hyde,[98] who seems rather inclined toward the sebaceous origin of the tumor and says that the psorospermic theory has been abandoned. He places the statistical frequency of the disease in America at 1.65 per 1,000.

And finally to epitomize the findings of literature let me append the following tables to show at a glance the attitude of the many distinguished investigators who have worked upon this puzzling problem:

Believers in the Follicular or Sebaceous Origin of the Tumor.

Eramus Wilson,	Lukomsky,	Ranvier,
Engel,	Walter Smith,	Renaut,
Rokitansky,	Fagge,	Sangster (?),
Bazin,	Vidal,	Crocker,
Virchow,	Rindfleisch,	Thin,
Hebra,	Biesiedecki,	Benda,
Tilbury Fox,	Hutchinson,	O. Israel,
Kaposi,	Startin,	Zeissl,
	Piffard.	

Believers in the Rete Origin of the Tumor.

Klebs,	C. Boeck,	Campana,
Von Bärensprung,	Lukomsky,	Török,
Bizzozero,	Sangster (?),	Tommasoli,
Manfredi,	Morrison,	Stanziale,
O. Simon,	Neisser,	H. von Hebra,
Retzius,	Caspary,	Kromayer,
Lostorfer,	Geber,	Unna,
	Audry.	

Believers in the Contagiousness of the Disease.

Bateman,	Ebert,	Majocchi,
Henderson,	Bizzozero,	Angelucci,
Patterson,	Manfredi,	Neisser,
Carswell,	Retzius,	Stelwagon,
Cotton,	C. Boeck,	Allen,
Caillault,	Liveing,	Török,
Klebs,	Vidal,	Tommasoli,
Hardy,	Mackenzi,	Graham,
Virchow,	Bollinger,	Kuznitzky.

Believers in the Non-contagiousness of the Disease.

Eramus Wilson,	Hebra,	G. H. Fox,
Von Bärensprung,	G. Simon,	Robinson.
Rokitansky,	Kaposi,	

Believers that the Bodies are Degenerated Epithelial Cells.

Auspitz,	Manfredi,	Török,
Virchow,	Vidal,	Tommasoli,
C. Boeck,	Renaut,	Stanziale,
Lukomsky,	Caspary,	Unna.
Bizzozero,	Geber,	

Believers that the Bodies are Parasites.

Klebs,	Neisser,	Benda,
Hardy,	Angelucci,	Mansuroff,
Retzius,	Pick,	Shaw.
Bollinger,	Touton,	

My personal observations relate to the study of several hundred sections cut from tumors sent me by Dr. Stelwagon of Philadelphia and by Dr. Shepherd of Montreal, to whom I wish to express my most sincere obligations. These tumors were hardened in alcohol, in Müller's and in Zenker's fluid. They were cut in paraffin, three and one-third micromillimeters in thickness, and stained by the following methods:

Hematoxylin eosin.
Iron-hematoxylin-acid fuchsin-picric acid.
Alkaline methylin blue-eosin.
Polychrome methylin blue-glycerine ether.
Picrocarminate of ammonia.
Gram.
Van Gieson.
Acid orcein-polychrome blue-orange tannin.
Thionin-eosin.
Gabbet-Ernst.
Ranvier's picrocarmin.
Sanfelice.
Gram and Bismark brown.
Neutral orcein-wasserblau, and
Carbol fuchsin-tannin-wasserblau.

It was not the object of this investigation to repeat many of the tests which other workers have made, such as the physical and chemical experiments of Török and Tommasoli, — these were accepted as facts, — but to observe and to interpret as carefully as possible the microchemical reactions of these individual sections after subjection to the above enumerated dyes.

THE TISSUE ADJACENT TO THE TUMOR.

Stratum Corneum. — The appearances in this layer vary considerably in the various sections. In places there is marked coherence of the cells, increased thickness of the layer, and persistence of the nuclei — parakeratosis; in other sections the layer seems normal and consists of a few thin lamellæ, wavy and rather dehiscent.

Stratum Lucidum. — Apparently absent in all sections.

Stratum Granulosum. — This layer is everywhere abnormal. In places we see it apparently fused with the horny layer, forming a tissue which receives the acid stains and containing occasional remnants of kerato-hyalin granules and disintegrated nuclei which absorb nuclear dyes but faintly. In places the layer is very thin, while in others it is distinctly

hyperplastic and contains swollen cells with large nuclei and very prominent kerato-hyalin granules. Finally we note a phenomenon which is very marked in all sections in the rete, namely, a clear halo about the nuclei formed by a shrinking of the perinuclear protoplasm. This is not caused by alcohol, for it is equally prominent in sections fixed in other hardening agents.

Stratum Spinosum. — Here again sections vary in detail, but are all abnormal even at considerable distance from the tumor. In places the palisade layer is composed of thickly grouped, irregularly shaped nuclei, but more often we note the entire absence of nuclei from this layer and find it difficult to follow the line of demarcation between the corium and the rete. The prickles are retained throughout. Above the palisade layer we begin to perceive the chief characteristic of the rete, that is, the shrinking of the cytoplasm leaving a clear, empty zone about the nuclei. Usually the nuclei remain round, but at times they are pressed against the side of the cell and become crescentic or again apparently disappear entirely. In some sections these nuclei are enormous. Usually there is a distinct absence of nucleoli, but in some sections these bodies are quite abundant and one can find six or seven in a single nucleus. As a rule, there is distinct acanthosis, but as the tumor is approached the layer fades away to the depth of a few cells. At times the whole rete stains poorly and then the different layers lose their identity and run into each other.

Before leaving this layer I wish to emphasize again the condensation of the cytoplasm from about the nucleus — a condition practically always present in these sections and yet apparently seldom alluded to in literature.

Corium. — It is practically impossible to describe in a few words the characteristics of this portion of the skin, for the picture varies with each tumor. The only constant features are the distinct diminution in the size and number of the papillæ, the moderate dilatation of the superficial vessels, and the decided infrequency of follicular and glandular structures.

The texture of the corium ranges from the most delicate

tracery of collagen with its normal nuclei to the densest masses of a-nuclear swollen tissue which appears from microchemical tests to be collacin. This rather uncommon substance appears most abundantly in two cases, and in fact with the exception of the tissue immediately below the rete and above the vessels seems to constitute the greater portion of the corium. As a rule, the corium shows marked signs of inflammatory reaction and the cells are grouped about the vessels and around the sebaceous glands whenever these are present in the section. Usually the cells are lymphocytes, but in one or two cases there is an unusual abundance of mast cells above the hair follicles and to a lesser extent about the lower row of horizontal vessels. In some sections there is marked œdema and the cell nuclei are, in consequence, much increased in size, while the protoplasm seems to have disappeared.

THE TUMOR PROPER.

The description which follows is a composite picture gleaned from the close study of many sections. Sometimes this curious metamorphosis can be clearly followed step by step through half a hundred layers of cells, sometimes it all occurs within a very few strata, but in all cases, unfortunately, the underlying cause has totally escaped us. Bacteria were nowhere to be found.

The new growth is formed by a hyperplasia of the rete cells which push the mass downwards and outwards, thus producing a globular tumor. Very often it would appear that the growth was the result of the combination of two or three down-buddings of the rete Malpighi, bringing about unusual appearances which will be alluded to later.

The lowest layer of cells in the tumor present all the characteristics seen in the quasi-normal spinous cells described above, when considering the adjacent epidermis, plus the appearance of well-marked nucleoli which are usually single or double, but may reach the number of three or four. These bodies stain very deeply when exposed to methylin-blue and are practically always present. In the midst of these cells

we find others devoid of nucleus and composed or fine fibrillary protoplasm. The spinous processes of all these cells are retained.

Above these primary rows we find cells which have lost some of their normal attributes. Their nuclei have become distorted and assume many shapes, but are still surrounded by the empty halo so characteristic of this process. Adjacent cells lose their nuclei and become unbounded masses of reticulated protoplasm, receiving only the basic colors.

After this so-called secondary stage the field becomes more difficult to interpret. Many cells increase enormously in size; assume many shapes, chiefly through pressure; the nucleus becomes flattened and apparently pressed to one end of the cells, but remains always surrounded by its empty zone; the cytoplasm grows more and more reticulated and its fibrillæ simulate the walls of a honeycomb with irregularly placed trabeculæ which still absorb the nuclear dyes. This reticulation apparently begins in the centre of the cell and spreads outward toward the periphery. The nucleus may be found near the upper or near the lower pole of the cell, or may be entirely wanting. Karyokinetic figures are often seen. In the midst of these large cells we frequently find others which have preserved their identity to a greater extent. Here the nuclei are large and the protoplasm, like that of their cells, is finely granular, and receives but faintly the coloring agent.

Still further toward the free surface we find other changes. Here the nuclei and their surrounding halos are, for the most part, gone, and the whole cell, now somewhat smaller in size, assumes the appearance of a multilocular cyst in which the trabeculæ absorb the basic and their contents, the acid dyes.

The next change is a very abrupt one and apparently occurs without our being able to detect its steps, and here, for the first time, we see a field where all elements are apparently contemporaneous. From the distinct evidences of kerato-hyalin granules about these new-formed masses, it is perhaps proper to infer that we are now in the stratum gran-

ulosum, and above this granular area the cells become much smaller, trabeculæ disappear from the cytoplasm, and we see a homogeneous mass which, in its lower layers, seems at times to be impartial to the dye it selects — enclosed by walls which suggest a close relationship to keratin near the free surface. These break, leaving ample opportunity for the bodies to escape.

Where the tumor consists of two or more lobules, which have finally approximated, leaving perpendicular walls of cells of the first-row type still clearly visible, we can trace this line of demarcation steadily upward. Curiously enough, these cells do not undergo the strange metamorphosis just described, but pass through the typical changes seen under normal conditions, resulting finally in vertical septa of keratin.

We have thus described the successive changes as they have appeared to our eyes, and it is interesting to note in how many important points we differ from other observers. We have never seen any bodies which we could possibly call gregarins. We have never seen any division of a nucleolus, and to our mind the nucleus and the nucleoli (plasmosomata) play no part whatever in the subsequent changes. We have never found any membrane about the cytoplasm walling off the nucleus and thus creating a doubly-contoured body. On the other hand, we find a marked frequency of the empty peri-nuclear space in the Malpighian and granular cells, and in most cases a definite inflammatory reaction about the tumor which, in two instances, went so far as to cause most emphatic colloid (?) degeneration of the major part of the corium.

On examining carefully the cells of the completed process, that is, the end result of this strange metamorphosis, and comparing their staining reaction with that of their envelopes and of the horny layer of the adjacent epidermis we are forcibly struck with their similarity. Out of eighty-five slides composed of nine hundred and twenty-eight sections there were only two slides where these staining reactions were not identical; in other words, these so-called molluscum bodies

are simply keratin, identical with the horny layer, except in the shape of the individual cells; and if we now review this strange process I think we must be convinced that these abnormal conditions cannot be properly considered a colloid or hyalin degeneration, for the staining reactions throughout the depth of the tumor are normal, but rather a very extraordinary metamorphosis of rete cells into normal keratin.

The bacteriological work of this question was undertaken by Dr. Robey, and his results are now given.

The great scarcity of fresh material has made the bacteriological study of molluscum contagiosum very unsatisfactory.

The tumors were excised with antiseptic precautions and immediately placed in tubes of bouillon and sealed with paraffin. Prior to the removal of the tumors from the tubes all dishes and instruments to be used in the process of inoculation were thoroughly sterilized. The tumor was cut open and the small bodies like swollen starch grains were squeezed and various culture media inoculated from their contents.

Bouillon.—A well-marked turbidity after twenty-four hours. Microscopic examination showed a pure culture of staphylococci.

Blood Serum. — After twenty-four hours there is a profuse growth of slightly moist, grayish white colonies about as large round as the ordinary platinum needle.

Agar-agar. — The growth was quite as profuse in twenty-four hours as on blood serum, but had a more moist appearance, since on blood serum after some inoculations the growth looked like fine flour dusted over the surface of the medium.

Glycerine Agar. — The same characteristics as on plain agar.

Gelatine Ten Per Cent. — After twenty-four hours a slight white growth along the course of the needle with liquefaction beginning as a cup-shaped depression, which slowly extended until the whole tube was liquefied after four or five weeks.

Transfer made at intervals of several days and weeks showed always a pure culture of the staphylococcus.

Special Skin Medium.— By means of a medium made from human skin we tried to produce artificially the habitat of the special bacterium or parasite of molluscum contagiosum. The portion of the skin used was made as free from fat as possible and was then finely chopped. The medium was made in the same manner as ordinary bouillon. The result was a milky fluid in which only a feeble growth of the staphylococcus occurred.

Media containing peptone, sodium chloride, and agar, as well as asparagin-agar were tried, but, due perhaps to some fault in technique, no growth was produced.

An anaerobic culture was made in glucose bouillon by Wright's method and a slight growth of staphylococci was obtained in forty-eight hours.

The organism found in these cultures stains by the ordinary anilin dyes and by Gram.

Bouillon emulsions were made from the contents of the tumors and inoculated into the abdominal cavity and skin of guinea-pigs. One pig died in ten days after inoculation with an abscess of the abdominal wall, cultures from which showed a pure growth of a staphylococcus indistinguishable in its characteristics from the staphylococcus pyogenes albus. Dr. White has examined the abscess wall histologically.

Four other pigs inoculated showed no change after several months.

The organism which has been found in this investigation is evidently the staphylococcus epidermidis albus of Welch, the normal inhabitant of the deeper layers of the skin.

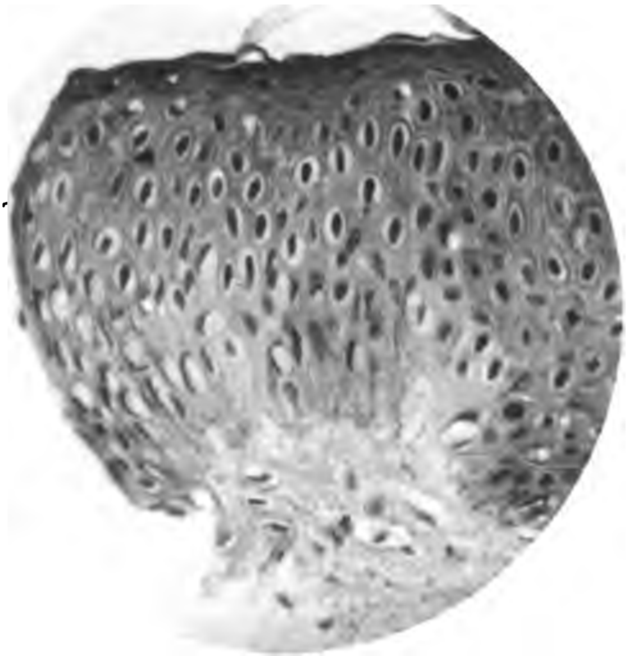
The results of the bacteriological examination of the tumors are negative and some new medium or stain must be devised to demonstrate the causative agent.

This completes the investigations undertaken by Dr. Robey and myself, and in conclusion I can only reiterate my belief that nobody has demonstrated up to this time any parasitic body in the growth, and that the change is not a colloid or hyalin degeneration, but rather an extraordinary metamorphosis of rete cells into keratin.

REFERENCES.

1. Bateman. 1817. Delineations of cutaneous diseases.
2. Cazenave and Schedel. 1828. Abrégé pratique des maladies de la peau.
3. Henderson. 1841. Edinburgh Med. and Surg. Journal, p. 213.
4. Patterson. 1841. Edinburgh Med. and Surg. Journal, p. 280.
5. Willis. 1841. Illustrations of Cutaneous Diseases.
6. E. Wilson. 1842. Diseases of skin, p. 341.
" 1863. Diseases of skin, p. 653.
7. Engel. 1844. Zeitschrift d. k. k. Gesellschaft der Aerzte in Wien.
8. Cotton. 1848. Edinburgh Med. and Surg. Journal, p. 82.
9. von Bärensprung. 1848. Beitrage zur Anatomie u. Pathologie der Haut, p. 43.
10. Rokitansky. 1856. Pathologische Anatomie, p. 79.
11. Caillault. 1859. Traité pratique des maladies de la peau chez les enfants, p. 54.
12. Klebs. 1859. Virchow's Archiv, p. 188.
13. Hardy. 1863. Leçons sur les maladies de la peau, p. 98.
14. Virchow. 1865. Berliner klinische Wochenschrift, p. 34.
15. " 1865. Virchow's Archiv, p. 144.
16. Neligan. 1866. Practical Treatise of Diseases of Skin, p. 305.
17. Ebert. 1870. Jahrbuch für Kinderheilkunde, p. 152.
18. Bizzozero and Manfredi. 1871. Archiv für Dermatologie, p. 599.
19. Retzius. 1871. Deutsche Klinik. No. 50.
20. Lostorfer. 1871. Archiv für Dermatol. und Syphilis, p. 184.
21. Retzius. 1872. Deutsche Klinik, p. 39.
22. Hebra. 1872. Lehrbuch der Hautkrankheiten.
23. Liveing. 1872. British Medical Journal, p. 11.
24. Squire. 1872. British Medical Journal, p. 45.
25. T. Fox. 1872. British Medical Journal, p. 538.
26. Eames. 1872. British Medical Journal, p. 680.
27. Ferrier. 1872. British Medical Journal, p. 682.
28. T. and C. Fox. 1872. Transactions Pathological Society, London, p. 460.
29. Boeck. 1875. Vierteljahresschrift für Dermatol. und Syphilis, p. 23.
30. Lukomsky. 1875. Virchow's Archiv, p. 145.
31. Bizzozero and Manfredi. 1876. Archiv für Dermatologie u. Syphilis, p. 610.
32. O. Simon. 1876. Vierteljahresschrift für Dermat. u. Syphilis, p. 400.
33. Kaposi. 1877. Vierteljahresschrift für Dermat. u. Syphilis, p. 333.
34. G. H. Fox. 1878. Chicago Medical Journal. May.
35. Barnes. 1878. British Medical Journal, p. 335.
36. Walter Smith. 1878. Dublin Journal of Medical Sciences. Nov.
37. Liveing. 1878. Lancet, p. 495.
38. Hutchinson. 1878. Lectures on Clinical Surgery, p. 1.

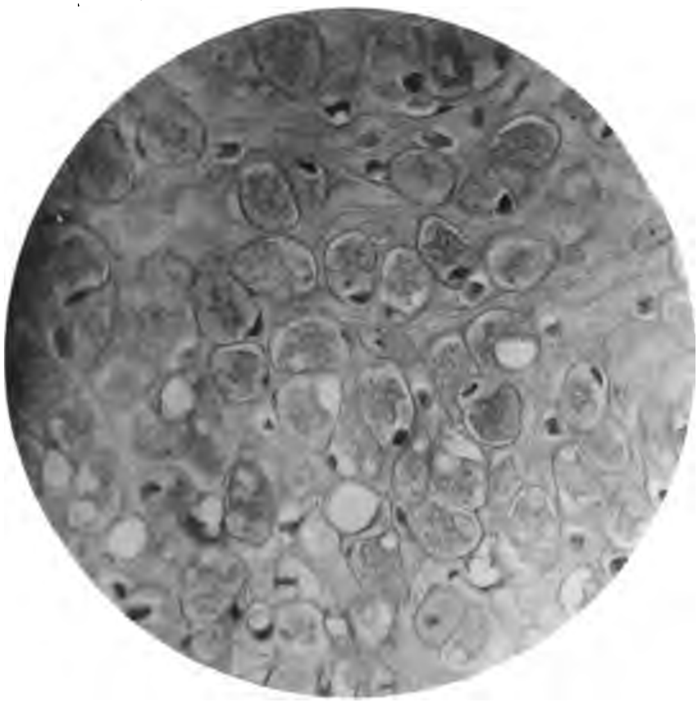
39. Vidal. 1878. Le Progrès Medical, p. 478.
40. Rindfleisch. 1878. Lehrbuch der pathologischen Gewebelehre, p. 284.
41. Mackenzie. 1879. British Medical Journal, p. 855.
42. Baker. 1879. British Medical Journal, p. 856.
43. Malcolm Morris. 1879. British Medical Journal, p. 856.
44. Brown. 1879. British Medical Journal, p. 856.
45. Bollinger. 1879. Vierteljahresschrift für Dermatologie und Syphilis, p. 52.
46. Startin. 1880. Lancet, p. 564.
47. Sangster. 1880. British Medical Journal, p. 327.
48. Majocchi. 1880. Gaz. med. di Roma, p. 237.
49. Brochin. 1880. Gazette des Hôpitaux, p. 1009.
50. Renaut. 1880. Annales de Dermatologie et de Syphiligraphie, p. 397.
51. Crocker. 1881. British Medical Journal. January 15.
52. Thin. 1881. Journal of Anatomy and Pathology, p. 202.
53. Auspitz. 1881. System der Hautkrankheiten, p. 139.
54. H. & B. Morrison. 1881. Medical Times. May.
55. Angelucci. 1881. International Medical Congress.
56. Neisser. 1882. Monatshefte für praktische Dermatologie. March.
57. Hebra. 1882. Monatshefte für praktische Dermatologie, p. 270.
58. Laache. 1882. Nordiskt Medicinskt Archiv. Nov. 21.
59. Caspary. 1882. Vierteljahresschrift für Dermat. u. Syph., p. 205.
60. Geber. 1882. Vierteljahresschrift für Dermat. u. Syph., p. 403.
61. Csoker. 1883. Vierteljahresschrift für Dermat. u. Syph., p. 628.
62. Campana. 1886. Giornale ital. delle Mal. ven. e della pelle., p. 1.
63. Haab. 1886. Correspondenzblatt für Aerzte. Nov. 8.
64. Allen. 1886. Jour. Cut. and Genit.-Urin. Diseases, p. 238.
65. Havenith. 1887. Journal de Medicine de Bruxelles. March 5.
66. Neisser. 1888. Vierteljahresschrift für Dermat. u. Syph., p. 553.
67. Stelwagon. 1889. Journal Cutan. and Gen. Urin. Diseases, p. 60.
68. Allen. 1889. New York Medical Record, p. 117.
69. Török and Tommasoli. 1890. Monatshefte für praktische Dermatologie, p. 149.
70. Stanziale. 1890. Giornale Internazionale delle Scienze Mediche, p. 321.
71. Allen. 1891. Transactions American Dermatological Association, p. 62.
72. Wigglesworth. 1891. Transactions American Dermatological Association, p. 63.
73. Jackson. 1891. Journal Cutaneous & Genito-Urinary Diseases, p. 338.
74. Pick. 1892. Ergaenzungsheft zum Archiv für Der. u. Sy., p. 89.
75. Graham. 1892. Journal Cutan. and Gen. Urin. Diseases, p. 89.
76. Macallum. 1892. Journal Cutan. and Gen. Urin. Diseases, p. 93.
77. Pick. 1892. Monatshefte für praktische Dermatologie, p. 133.
78. Neisser. 1893. Verhandlungen der deutschen dermatologischen Gesellschaft. IV. Congress, p. 589.



1

2

White. Molluscum Contagiosum.



3

4

White. Molluscum Contagiosum.

79. Nobel. 1893. Archiv. für Dermatologie und Syphilis, p. 929.
80. Crocker. 1893. Diseases of skin, p. 479.
81. Neumann. 1893. Archiv für Dermatologie und Syphilis, p. 980.
82. Hansemann. 1894. Berliner Klinische Wochenschrift. Nov. 1.
83. Stelwagon. 1895. Journal Cutaneous & Genito-Urinary Diseases, p. 50.
84. Kuznitzky. 1895. Archiv. für Dermatologie und Syphilis, p. 65.
85. Touton. 1895. Archiv für Dermatologie und Syphilis, p. 369.
86. Benda. 1895. Zeitschrift für Dermatologie, p. 195.
87. Beck. 1896. Archiv für Dermatologie und Syphilis, p. 167.
88. Unna. 1896. Lehrbuch (Walker's Translation), p. 795.
89. Gaucher et Sergent. 1898. Archive de Medicine Expérimentale. September.
90. Audry. 1899. Annales de Dermatol. et de Syph., p. 621.
91. Hallopeau et Leredde. 1900. Dermatologie, p. 316.
92. Joseph und Loewenbach. 1900. Dermatologischer Tecknik, p. 74.
93. Hyde. 1901. Diseases of Skin, 5th Edition, p. 467.

DESCRIPTION OF PLATES XIX AND XX.

FIG. 1. — Shows (*a*) absence of stratum germinativum; (*b*) indistinct demarcation between corium and epidermis; (*c*) perinuclear halo, irregularity in size of lower nuclei and their frequent total disappearance; and (*d*) distinctly abnormal keratinization in stratum granulosum and stratum corneum.

FIG. 2. — Shows (*a*) partly normal lower layer of cells with perinuclear halo; (*b*) on the left, approximation of two lobules of epidermal tissue with suggestion of normal metamorphosis of cells above; (*c*) pushing of nuclei to one side with formation of honeycomb reticulation and great hyperplasia of cell; (*d*) loss of nuclei from certain cells; and (*e*) persistence of quasi-normal cells throughout the field.

FIG. 3. — Presents condition of metamorphosis just below level of granular layer, showing (*a*) more advanced stage of process; (*b*) appearance of completed state of reticulation with absence of nuclei and decreased size of cell; and (*e*) persistence of quasi-normal cells.

FIG. 4. — Presents the metamorphosis completed, showing the swollen homogeneous keratin bodies with their keratin envelopes.

CULTURE EXPERIMENTS WITH MALIGNANT TUMORS, 1900–1901.

OSCAR RICHARDSON, M.D.
(*Clinico-Pathological Laboratory, Massachusetts General Hospital.*)

In continuation of our report for 1899–1900 of the research work done in this Laboratory for the Cancer Investigation Commission in regard to culture experiments with carcinomatous tissue, we have now to record the results of a series of culture experiments with tissues and fluids from malignant tumors made during the past year.

The cultures were made from material obtained in this Hospital, and in most of the cases they were made as soon as possible after the operation.

The method of taking the culture material from the new-growth is essentially that detailed in our last report, excepting that we now use a glass tube with a rubber bulb at one end, which enables one, by pressing on the bulb and introducing the free end of the tube into the curetted pulpy semi-fluid mass of the new-growth, to suck up into the tube any desired quantity of the material. The tube and bulb are easily sterilized.

In this manner and following out as well the technique laid down in our previous report the liability of contamination is reduced to a minimum.

Media.

We have to mention as additions to the list previously reported the following:

Bouillon and agar made from human liver.

Tartaric acid glucose bouillon. (Recommended by Sanfelice for the cultivation of so-called blastomycetes.)

Litmus milk.

Dunham's pepton solution.

Methods of Cultivation.

We have nothing to add to our previous report, except that in certain instances we have used larger quantities of the culture media.

The records of the details of the culture experiments are to be found in the accompanying table.

The cultures recorded in the table were observed over varying periods of time, all of them, except where noted, being observed for fifteen days at least and many of them for months. They were subjected to regular macroscopical and microscopical examinations, and from many of them sub-cultures were made.

The result of our investigations confirms the report of our previous experiments that we are unable to cultivate from the tissues and fluids of malignant new-growths anything which can be regarded as a specific infecting organism.

FOUR PATHOGENIC TORULÆ. (BLASTOMYCETES.)

JOSEPH D. WEIS, M.D.

(Austin Teaching Fellow in Bacteriology in Harvard University.)

(From the Bacteriological Laboratory of the Harvard Medical School.)

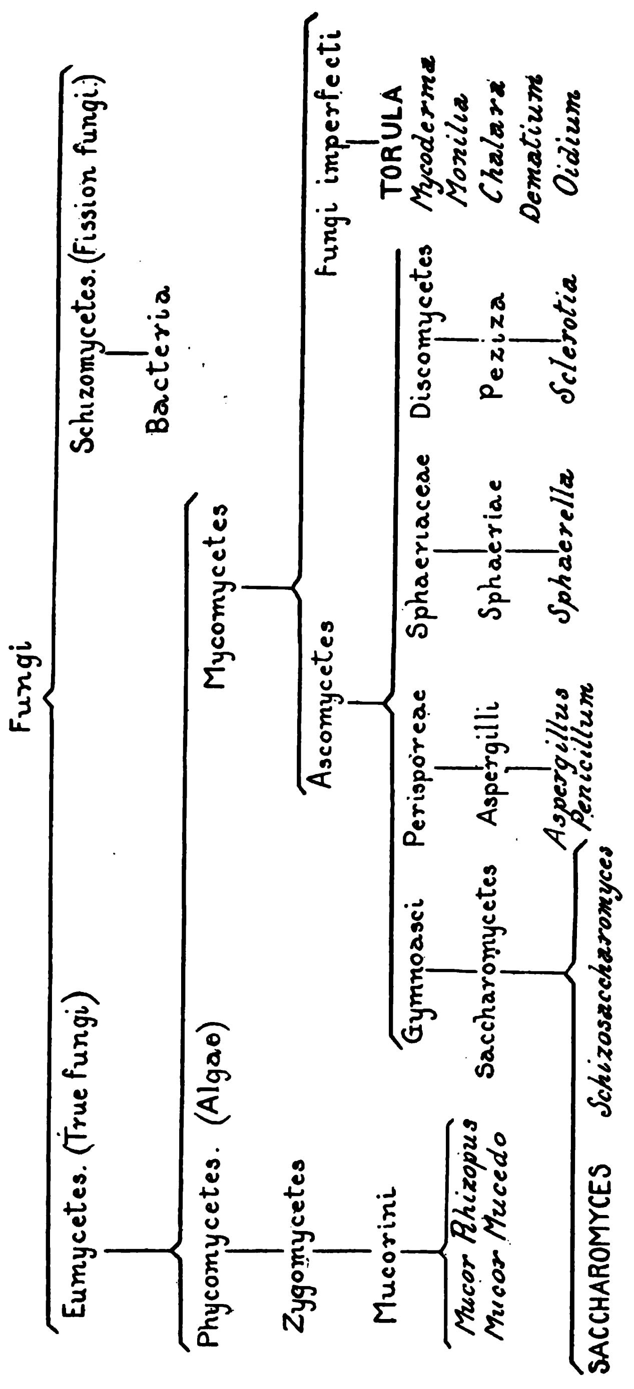
The idea that malignant tumors are caused by parasites has become widespread among medical men during recent years. Among the various kinds of micro-organisms which have been regarded as the infectious agents are certain fungi which have certain similarity to the yeasts and to which the name of Yeasts and Blastomycetes has been given. Thus Sanfelice and Plimmer claim to have isolated blastomycetes from carcinoma and sarcoma, and with his blastomycetes Sanfelice has been able, by inoculation in various animals, to produce what to his mind are malignant tumors.

In view of the importance thus assigned to these micro-organisms in connection with the etiology of malignant tumors, it has seemed desirable to make a systematic study of as many of them as possible by the special methods used in the technical study of yeasts, in order to determine their proper place in the modern classification of yeasts and allied fungi.

For this study I have been fortunate enough, through the kindness of the observers themselves, to procure cultures of the blastomycetes or yeasts of Professor Sanfelice, Dr. Plimmer, and Professor Klein.

I have chosen from many specimens four different ones, two of which had been isolated directly from malignant tumors. One, though obtained from fruit, is said to be able to cause cancer; and that of Professor Klein which, though having no relation whatever to cancer, is, however, pathogenic for guinea-pigs and is so obviously of the same nature that I thought it instructive to study it in comparison with the other three.

Before detailing the results of the study of these micro-organisms, it will conduce to a better understanding of the


Fungi
Eumycetes. (True fungi)
Schizomycetes. (Fission fungi.)
Bacteria
Phycomycetes. (Algae)
Mycomycetes
Zygomycetes
Mucorini
Mucor Rhizopus
Mucor Mucedo
Ascomycetes
Fungi imperfecti
Gymnoasci
Perisporeae
Sphaeriaceae
Discomycetes
Saccharomycetes
Aspergilli
Sphaeriae
Peziza
Aspergillus
Penicillium
Sphaerella
Sclerotia
SACCHAROMYCES
Schizosaccharomyces
TORULA
Mycoderma
Monilia
Chalara
Dematium
Oidium

subject to point out what place the yeast-like micro-organisms in general occupy among the fungi.

The classification of the fungi which I adopt is that given by Klöcker of Copenhagen, and accepted by Hansen and Jörgensen. It is by no means complete and is probably not generally accepted in its entirety by cryptogamic botanists in that it is too general, but for our purposes and for the purposes of the scientific brewing, distilling, and yeast manufacturing industries it is sufficient.

The table given on page 281 is taken from Klöcker's "Die Gärungsorganismen." The fungi are here divided into two large groups — Eumycetes and Schizomycetes. The Eumycetes are the true fungi. The Schizomycetes are the bacteria. The true fungi are further divided into two large divisions — Phycomycetes (Algæ) and the higher fungi, the Mycomycetes. With the former we have to deal only with the Zygomycetes or bridge fungi, an example of which are the Mucorini. There are then left the Mycomycetes, which are characterized as a class by the fact that their filaments (or the mycelium), when present, are divided by transverse walls or septa.

These Mycomycetes are divided by Klöcker into the Ascómycetes and the Fungi Imperfecti. Under the Ascomycetes are classified the fungi which produce endospores in a sporangium which is called the ascus. The character of this ascus qualifies the four groups as follows:

The Gymnoasci with the family Saccharomycetes, or the yeasts.

The Perisporeæ with the family Aspergilli.

The Sphæriacea with the family Sphæriæ.

The Dyscomycetes with the family Peziza.

Under the first, the Gymnoasci, the ascus is in its simplest form; that is, the ascus or germ-tube has no tissue-life covering, hence the name gymnoascus or naked ascus. Under these are classed the saccharomycetes or yeasts.

The second division, the Perisporeæ, are characterized by having the ascus or germ-tube, containing the ascospores, itself enclosed within the so-called perithecium or pyreno-

carp (a round cavity with a wall of one or more cells in thickness and entirely closed).

The third division, the Sphæriaceæ, on the other hand, and in contrast to the perisporeæ, have a mouth or aperture in the ascocarp, and the perithecia lie in the superficial layers of the organism and end in simple, rarely papilla-shaped openings.

The fourth division, the Discomycetes, are characterized by an open ascocarp, or germ-tube.

Under the Fungi imperfecti are classed many fungi which have never been observed to show the special characteristics of any of the four groups of the ascomycetes, but yet are to be placed among the mycomycetes. Hence they have been classed together as imperfect fungi, or as Klöcker very graphically puts it, "have been shelved in a lumber room" ("in einer Rumpelkammer zusammengestellt"). In this lumber room are to be found, among others, such as the oidia, mycoderma, etc., certain forms that are named Torulæ.

The name Torula has been given to many different organisms, but by Hansen, the greatest authority on the subject, and by Klöcker, it is restricted to those micro-organisms which are similar to Saccharomycetes or yeasts, but which do not produce spores and do not develop any mould-like vegetation (mycelia).

With regard to alcohol production, torulæ may show any grade of fermentative power. Hansen believes from this that, with further knowledge and research, many of these high fermentative forms will be found to be separate and distinct from the great mass of the torulæ which in general have no fermentative power, or have it in such slight degree as to be almost unrecognizable.

There are torulæ known which have all the physiological and morphological characters of saccharomycetes, except the most important morphological attribute of the saccharomycetes, the endospore production, which, according to the classification, never occurs among the torulæ. Indeed, so artificial is the classification here that if a given torula should in the history of its cultivation suddenly produce spores, it

would at once cease to be classed as a torula and become henceforth a saccharomycetes.

It has been well shown by Hansen that a saccharomyces does not always show the same form or shape; that is, there are always variations. For some unknown reason these organisms, therefore, are not constant in form, nor, for the matter of that, are they constant in manner of growth. But the general features are constant enough to distinguish one from another. We cannot, therefore, determine with which organism we are dealing by the consideration of its morphology alone.

Torulæ are widely distributed in Nature, according to Klöcker. Hansen found them always in the earth and in large numbers on the surface of and in the nests of bees, wasps, and other insects. In the spring they are easily obtained in the air, especially under the boughs of trees and the branches of plants.

A superficial examination of all four organisms which I have studied shows that they must be classed either in the first group of the ascomycetes, the gymnoasci, family *saccharomycetes*, or among fungi imperfecti, family *torulæ;* in other words, are these organisms true yeasts, as has been claimed by some observers, — that is, do they produce spores and do they ferment sugars, or are they destitute of these characteristics and therefore to be regarded as torulæ ?

As has been mentioned above, the name Blastomycetes has also been given to these organisms. While this term has no place in the classification of Klöcker, according to Gotschlich it means any organism which reproduces itself by budding.

In a general classification given by Gotschlich the lower fungi are divided into four groups:

[1] 1. The thread (*Faden*) or mould fungi (Hyphomycetes).

2. The budding (*Spross*) or yeast fungi (Blastomycetes).

3. The Streptothrices (Branching Bacteria).

4. The fisson (*Spalt*) fungi or bacteria (Schizomycetes).

[1] Words in italics are German.

With this classification we have only to do with Number two, the *Sprosspilze* or budding fungi — the Blastomycetes.

In this classification, therefore, we see that the term Blastomycetes means simply an organism which reproduces itself by the budding process and does not grow out into filaments and produce a true mycelium.

Under this head we must therefore group Yeasts and Torulæ.

Yeasts or saccharomycetes are distinguished first then by being Blastomycetes. But all saccharomycetes must show two invariable characteristics, namely, the power of fermenting sugars and the power of endogenous ascospore formation; that is, the formation of spores within an ascus. Any blastomyces, therefore, which under no circumstances shows either of these characteristics must be thrown out of the saccharomycetes group.

These non-fermenting asporogenous blastomycetes are the torulæ.

This statement is not absolutely true, and needs some qualification. I have stated above that certain torulæ do have fermentative power of high degree. This is true, but they are very rare. As a general rule, therefore, torulæ do not cause fermentation of any of the sugars, and this is so universal that the fermentation of sugar by a few torulæ has caused Hansen to question their classification among the torulæ.

To sum up, therefore:

Blastomycetes include both the saccharomycetes and the torulæ.

The saccharomycetes are fermenting, endosporogenous blastomycetes.

The Torulæ are asporogenous blastomycetes with or without the power of fermentation.

The term Blastomycetes has perhaps been well applied to the group of pathogenic organisms about to be considered. But by medical men in general the term has been considered to be synonymous with yeasts (Saccharomycetes).

As a result of my investigation I have come to the con-

clusion that this idea is erroneous and that these organisms are not yeasts but rather belong to the group torula, which, as has been shown above, is a distinct and separate group in the present classification of cryptogamic botanists.

The method of reproduction of blastomycetes (Saccharomycetes and Torulæ) is distinct from that of bacteria or of Schizosaccharomycetes. In the case of blastomycetes there is reproduction from the mother cell. The daughter cell may then be separated from the mother, or, as seen in the moist chamber, remain in connection with the mother cell and, itself becoming a mother cell, make one of the links in the chain of growth of a colony. In bacterial growth there is reproduction by fission. In blastomycetes this process never occurs; when occurring, it forms the class Schizosaccharomycetes.

This sporulation which is seen in true saccharomycetes has seemingly a double function. First a method of self-preservation under adverse circumstances, and later when proper conditions for growth are present, there takes place a rupture or disappearance of the original cell wall, and the spores, being set free, are able to take on the function of reproduction with formation of the cells of the species.

The morphological characters of the torulæ do not vary from those of the saccharomycetes, excepting, of course, in the spore production.

The cells may be round, oval, or even sausage-shaped, usually round, however. They vary also greatly in size and all variations are seen in the same culture. The cell consists of a mass of protoplasm enclosed in a membrane. This is its youngest and simplest form. The existence of a nucleus is uncertain. Frosch believes that it has unquestionably been demonstrated in some saccharomycetes, but not in all. Indeed, opinions differ widely: some, as Janssens and Leblanc, have seen two nuclei in one cell, after a very long hardening process with iodine and iodide of potash. Moeller, Hansen, and others claim to have seen but one, that also after prolonged hardening process. I have seen no statements anywhere with regard to the nuclei in torulæ.

The protoplasm is a fine network which, in the case of saccharomycetes, changes during fermentation. In the protoplasm of all blastomycetes, both torulæ and saccharomycetes, are to be seen vacuoles and oil drops, even in the very youngest forms. There is often seen in the vacuole a highly refractive body, the so-called vacuole-nucleus, which shows brownian motion. The vacuoles vary from one to three or more in number in one cell, and are filled with a fluid named by the Germans Zellsaft (cell juice). In the protoplasm are large and small granules, often square in form. The granular protoplasm is soluble in alcohol, ether, and other solvents. Will claims them to be of fatty nature. This observer claims to have seen small empty bubbles in which was a fine network after handling with absolute alcohol. By the addition of alcohol it is possible to see the cells shrink markedly. Dead cells are stained much more easily than living cells.

The membrane of the cell is very thin in young cells. In older cells, or cells under unfavorable circumstances, this membrane becomes very thick. By the application of dilute acids or alkalies, the membrane may be made very distinct.

According to Casagrandi and Will, the membrane has two or more layers. It is easily visible after treatment with one per cent osmic acid and is soluble in concentrated chromic or sulphuric acids. The membrane has the power, under certain circumstances, to produce a slimy gelatinous substance. Hansen has named this the "Gelatinous network."

Hardening of the usual microscopic preparation shows this network to be made up of strings and plates within which the cells lie. Many cells fall out of this network and leave empty spaces. This gelatinous network is to be seen on the surface of plaster of Paris blocks in cultures for spores and under other conditions. The source of this substance is yet a disputed question. Klöcker sums up the many theories: "It is very difficult at present to differentiate what, in the making up of the network, comes from the cell wall itself and what from the cell contents and what from the media. Perhaps this is a problem which at present is not solvable."

This formation of a substance about the cells is of importance and interest because in experimental inoculations there is to be seen within the tissues around the micro-organisms a definite halo or area which is unstained. It would seem that probably this gelatinous network may account for this appearance.

In old cultures on solid media of one of the organisms studied by me, I have often seen this material around individual cells, seemingly broken off from the original network. (Plate XXI., Fig. b, and Plate XXII., Fig. a.)

It is not within the scope of the present paper to give a history of the micro-organisms of fermentation, nor a detailed account of the technique taught by Jörgensen and Hansen, but it is enough to say that the history is intimately connected with the names of Pasteur and Hansen, to the latter of whom we owe the pure culture yeast breweries, distilleries, and bread yeast industries. The technique of this work at first sight is very complicated, but on closer acquaintance it proves to be not radically different from that of bacteriological work.

Each culture is absolutely pure in a sense truer than in bacteriology, in that each culture was grown from a single cell.

After these single cell colonies were developed the method of growth was followed by means of the Moist Chamber. The appearances upon different media were observed in Freudenreich flasks, except in the case of blood serum, milk, bouillon, and potato.

I have followed out the methods and principles of Hansen and Jörgenson, namely, those of the school of Copenhagen.

The cultures as stated above were obtained directly from the investigators who first isolated them. From a number of different specimens I have chosen these four:

1. The blastomyces of Sanfelice, isolated by him from an adenocarcinoma of the human ovary, which I have named T. Sanfelice.

2. The "Neoformans," a name given by Sanfelice to a blastomyces, first isolated from the surface of ripe peaches

and afterwards inoculated into animals with positive results by him.

3. A blastomyces found by Klein of London in milk which proved to be pathogenic for guinea-pigs, which I have named T. Klein.

4. Plimmer's blastomyces isolated by him from cancer of the breast, called in this paper T. Plimmer.

Staining has proven, in my hands, very unsatisfactory. It is practically impossible to obtain a permanent stained preparation of torula free from marked distortion and deformity. The process of drying and fixing by heat seems to contort and deform the organisms so much that at times they are almost unrecognizable. I have used many methods of fixation and find none satisfactory. Hardening of the smear in formalin or Zenker's fluid is also unsatisfactory. The best results were obtained by using Wright's modification of Jenner's blood stain.[1] By the use of this stain it is possible to get a fairly perfect picture of the cell; and also what is of great interest, there is to be seen within the protoplasm of the cell, separate and distinct from the vacuoles and oil drops, a round deeply staining body, well marked and unmistakable. This is without doubt to be considered the nucleus of the cell. With no other stain was it possible to observe this. The use of this stain is satisfactory, but it is not possible to transfer the cover-glass from water into balsam or any clearing media without great distortion occurring, and as, if left in water, the stain soon fades entirely, it is impossible to preserve the preparation for any length of time.

The detailed account of my observations of these organisms is given below. It will be apparent that all four organisms have almost the same characteristics and show but few variations one from another.

They do not ferment any of the sugars.

They do not form spores.

Under none of the conditions are mycelia seen.

It is interesting to note that although all four of these torulæ present many similar characteristics, there are some

[1] This Journal, Vol. VII., No. 1, p. 138.

TABLE I

				NEOFORMANS	SANFELICE	PLIMMER	KLEIN
MORPHOLOGY		Spherical		—	+	+	+
		Oval		← Occur in old forms	—	← Occur in old forms	—
		Size		2 μ to 20 μ	1 μ to 12 μ	2 μ to 10 μ	2 μ to 9 μ
	Protoplasm	Granular		—	+	+	+
		Vacuoles		+ (1 to 3)	+ (1 to 3)	+ (1 to 3)	+ (1 to 3)
		Oil drops		—	+	+	+
		Nucleus		—	+	+	+
		Mycelium		—	—	—	—
	Capsule	Young	Slight	— (very)	+	+	+ (very)
			Marked	—	—	—	—
		Old	Slight	—	—	—	+
			Marked	+	+ (very)	—	—
Gelatinous secretion				+	+	—	—
TEMPERATURE	Growth	Maximum		40° C.	40° C	40° C.	40° C
		Minimum		5° to 7° C.	5° to 7° C	8° to 10° C.	5° to 7° C.
		Optimum		24° to 25° C.	24° to 25° C.	24° to 25° C.	24° to 25° C.
	Life	Maximum					
		Minimum					
REPRODUCTION.	Moist chamber	Open budding		—	—	+	+
		Close budding.		+	+	—	—
	Resting cells.			+	+	+	+
	Ascospores.	On gypsum blocks. Observations extend over six months.	45° C.	—	—	—	—
			40° C.	—	—	—	—
			30°-35° C	—	—	—	—
			28°-30° C	—	—	—	—
			25° C.	—	—	—	—
			20°-22° C	—	—	—	—
			12°-15° C	—	—	—	—
			8°-0° C	—	—	—	—
FERMENTATION TESTS.	Yeast water.	Lactose	15° C.	—	—	—	—
			25° C.	—	—	—	—
			35° C.	—	—	—	—
			40° C.	—	—	—	—
		Dextrose	15° C.	—	—	—	—
			25° C.	—	—	—	—
			35° C.	—	—	—	—
			40° C.	—	—	—	—
		Saccharose	15° C.	—	—	—	—
			25° C.	—	—	—	—
			35° C.	—	—	—	—
			40° C.	—	—	—	—
	Wort		15° C.	—	—	—	—
			25° C.	—	—	—	—
			35° C.	—	—	—	—
			40° C.	—	—	—	—
Facultative Anaërobic.				+	+	+	+

TABLE. II.

					NEOFORMANS.	SANFELICE	PLIMMER.	KLEIN
FILM FORMATION. WORT	FREUDENREICH FLASKS.	Primary			−	+	−	+
		Secondary.			+	−	+	−
		Odor.			Of strong cheese.	Of strong stale cheese.	Of strong cheese	Of cheese.
		Observations extend over six months.		45° C.	−	−	−	−
				40° C.	6 to 8 days.	5 to 6 days.	8 to 10 days.	4 to 6 days.
				30°-35° C.	3 to 4 days.	2 to 3 days	1 to 2 days.	2 to 3 days.
				28°-30° C.	72 hours.	18 to 24 hours.	48 hours.	74 hours.
				25° C.	24 hours.	10 hours.	24 hours.	18 hours.
				20°-22° C.	1 to 3 days.	1 to 8 hours.	36 hours.	36 hours.
				12°-15° C.		3 to 4 days.	4 to 5 days.	72 hours.
				8°-10° C.	3 to 4 mos.	2 weeks.	4 to 8 weeks.	1 to 2 mos.
				5°-7° C.	−	1 to 2 mos.	−	2 to 3 mos.
				-5° C.	−	−	−	−
CULTURAL APPEARANCES.	TEST TUBES.	LIQUID.	Sediment	Slight.	−	+	−	+
				Heavy.	+	−	+	−
			Film	Marked.	−	+	−	+
				Slight.	+	−	+	−
		SOLID. Stab culture.	Surface	Flat, with slight elevation.	+	+	+	+
			Needle track	Stalactite form.	+	+	+	+
	FREUDENREICH FLASKS.	LIQUID.	Film.	Marked.	−	+	−	+
				Slight.	+	−	+	−
			Sediment	Fine powder	+	−	−	+
				Granular.	+	+	+	+
				Flocculent	−	+	+	−
			Clear supernatant fluid		+	+	+	+
			Cloudy on shaking.		+	+	+	+
			Yeast ring.		+	+	+	+
		SOLID.	Giant culture.	Flat at first.	+	+	+	+
				Elevated later.	+	+	+	+
				Beveled edges.	+	+	+	+
				Covers whole surface.	+	+	+	+
				Pasty	+	+	+	+
				Dirty yellow.	+	+	+	+
			Gelatin	Liquefaction.	+	+	+	+
				Non-liquefaction.	−	−	−	−
POTATO. COLOR.		Gray with translucent areas			+	−	−	−
		Yellowish brown.			−	−	+	+
		Dark brown.			−	+	−	−
LITMUS MILK.		Acid			−	−	−	−
		Alkaline			+	+	+	+
		Neutral			−	−	−	−
BEST CONDITIONS AND MEDIA.		Acid			+	+	+	+
		Alkaline			−	−	−	−
		Neutral			−	−	−	−
		Wort			+	+	+	+
		Lactose yeast water.			−	−	−	+
		Wort agar agar.			+	+	+	+
		Bouillon			−	−	−	−
		Potato			+	+	+	+
		Wort gelatin			+	+	+	+

TABLE I.

			NEOFORMANS.	SANFELICE	PL…
MORPHOLOGY.		Spherical	+	+	
		Oval.	(+) Occ. in old forms.	—	
		Size.	2μ to 20μ	1μ to 12μ	
	Protoplasm.	Granular.	+	+	
		Vacuoles.	+(1 to 3)	+(1 to	
		Oil drops.	+		
		Nucleus.	+		
		Mycelium	—		
	Capsule Young	Slight.	+(very)		
		Marked.	—		
	Capsule Old	Slight.	—		
		Marked.	+		
Gelatinous secretion			+		
TEMPERATURE.	Growth.	Maximum.	40°		
		Minimum.	5°		
		Optimum.	2[illegible]		
	Life	Maximum.			
		Minimum.			
REPRODUCTION.	Moist chamber.	Open budding.			
		Close buddi…			
	Resting…				
	Ascospores.	On gypsum blocks.			
…ON TESTS.	…ter.				

TABLE II.

NEOFORMANS. SANFELICE PL…

…cula

… Prof. J. Constan-
… Professor Metchnikoff
Prof. Francesco Sanfelice of
…dinia, to Dr. Carl Sternberg of
…nkenanstalt in Vienna, to Drs. Alfred
…hr. Holm of Copenhagen for instruction
…ny kindnesses and courtesies to me.

Explanation of Signs used in Table.

…ositive, present, or most favorable.

— Negative, not present, or least favorable.

In the experiments for ascospores and fermentation tests (Table I.) each organism was left to the temperature indi-

r a varying time. No observation is re-
s the given flask or gypsum block had
t six months; many were observed

e II., the number of hours, days,
he limit of time when the first
observed.

L DETAILS.

rvations it is nec-

meaning pel-
ked differ-

pro-
o vacu-
rence is to
at is to say, as-
wer of germinating
g analogous to cells con-

the growth of the organism upon
all, intimately adherent to the glass
ashed off by moving of the fluid.
cures" is meant a culture made upon solid
udenreich flasks after this manner: with a glass
ized in the flame, a drop of the fluid growth in Wort
ade to fall upon the surface of the gelatine or agar and
there allowed to grow.

By "bottom torula" and "top torula" is meant the characteristic of the organism of growing primarily either at the surface or at the bottom of the fluid. In "bottom growths" the top growth is secondary, and formed by the rising of cells to the surface. In "top growths" the bottom growth is secondary and is formed by the sinking of cells to the bottom of the flask.

marked differences. As for instance, Neoformans and Plimmer, though both "bottom torulæ," are to be differentiated in that Neoformans is a "close-budding torula," while Plimmer is an "open budding torula." And the same is true of Sanfelice and Klein, both "top torulæ;" but Sanfelice is a "close budding torula," while Klein is an "open budding torula." We have here a difference which is characteristic in each case. I have found in working with these organisms that they also showed the inconstancy and variability spoken of by Hansen and other observers. At first they varied markedly in their manner of growth on the same media at different times, but finally, after months of "cultivated life" they have settled down to fairly constant characters and forms.

From the fact that they do not ferment any of the sugars and that they do not form endospores, I have concluded that these organisms are not to be classed as saccharomycetes, but as torulæ, according to the principles laid down in the first part of this paper.

When we consider that torulæ of all sorts are found in the air and earth, it is not surprising to find that different observers have found similar, if not identical, species on their culture media, even when we know that one was found in milk, one on ripe fruit, and another on the surface of media which had been planted with cancer scrapings.

I wish to acknowledge my obligations to Prof. J. Constantin of L'École Normale Supérieure, to Professor Metchnikoff of L'Institut Pasteur in Paris, to Prof. Francesco Sanfelice of the Royal University in Sardinia, to Dr. Carl Sternberg of The Rudolf-Stiftung Krankenanstalt in Vienna, to Drs. Alfred Jörgensen and Just Chr. Holm of Copenhagen for instruction and for their many kindnesses and courtesies to me.

Explanation of Signs used in Table.

+ Positive, present, or most favorable.

— Negative, not present, or least favorable.

In the experiments for ascospores and fermentation tests (Table I.) each organism was left to the temperature indi-

cated in the table for a varying time. No observation is recorded, however, unless the given flask or gypsum block had been exposed for *at least* six months; many were observed up to the ninth month.

Under film formation, Table II., the number of hours, days, weeks, or months indicated is the limit of time when the first evidences of film production were observed.

MORPHOLOGICAL AND CULTURAL DETAILS.

To make clear the description of my observations it is necessary to make a few explanatory statements.

We speak of "film" and "bottom growth," meaning pellicle and sediment. Often here there may be marked differences in the cells.

By "resting cells" is meant cells with very refractive protoplasm, oil drops and thick cell walls containing no vacuoles. When present in a given organism the inference is to be made that no spores will be produced, that is to say, asporogenous. These cells have the power of germinating after long periods of inactivity, being analogous to cells containing spores.

By "yeast ring" is meant the growth of the organism upon the inside of the flask wall, intimately adherent to the glass and not able to be washed off by moving of the fluid.

By "giant cultures" is meant a culture made upon solid media in Freudenreich flasks after this manner: with a glass rod sterilized in the flame, a drop of the fluid growth in Wort is made to fall upon the surface of the gelatine or agar and there allowed to grow.

By "bottom torula" and "top torula" is meant the characteristic of the organism of growing primarily either at the surface or at the bottom of the fluid. In "bottom growths" the top growth is secondary, and formed by the rising of cells to the surface. In "top growths" the bottom growth is secondary and is formed by the sinking of cells to the bottom of the flask.

marked differences. As for instance, Neoformans and Plimmer, though both "bottom torulæ," are to be differentiated in that Neoformans is a "close-budding torula," while Plimmer is an "open budding torula." And the same is true of Sanfelice and Klein, both "top torulæ;" but Sanfelice is a "close budding torula," while Klein is an "open budding torula." We have here a difference which is characteristic in each case. I have found in working with these organisms that they also showed the inconstancy and variability spoken of by Hansen and other observers. At first they varied markedly in their manner of growth on the same media at different times, but finally, after months of "cultivated life" they have settled down to fairly constant characters and forms.

From the fact that they do not ferment any of the sugars and that they do not form endospores, I have concluded that these organisms are not to be classed as saccharomycetes, but as torulæ, according to the principles laid down in the first part of this paper.

When we consider that torulæ of all sorts are found in the air and earth, it is not surprising to find that different observers have found similar, if not identical, species on their culture media, even when we know that one was found in milk, one on ripe fruit, and another on the surface of media which had been planted with cancer scrapings.

I wish to acknowledge my obligations to Prof. J. Constantin of L'École Normale Supérieure, to Professor Metchnikoff of L'Institut Pasteur in Paris, to Prof. Francesco Sanfelice of the Royal University in Sardinia, to Dr. Carl Sternberg of The Rudolf-Stiftung Krankenanstalt in Vienna, to Drs. Alfred Jörgensen and Just Chr. Holm of Copenhagen for instruction and for their many kindnesses and courtesies to me.

Explanation of Signs used in Table.

+ Positive, present, or most favorable.

— Negative, not present, or least favorable.

In the experiments for ascospores and fermentation tests (Table I.) each organism was left to the temperature indi-

cated in the table for a varying time. No observation is recorded, however, unless the given flask or gypsum block had been exposed for *at least* six months; many were observed up to the ninth month.

Under film formation, Table II., the number of hours, days, weeks, or months indicated is the limit of time when the first evidences of film production were observed.

MORPHOLOGICAL AND CULTURAL DETAILS.

To make clear the description of my observations it is necessary to make a few explanatory statements.

We speak of "film" and "bottom growth," meaning pellicle and sediment. Often here there may be marked differences in the cells.

By "resting cells" is meant cells with very refractive protoplasm, oil drops and thick cell walls containing no vacuoles. When present in a given organism the inference is to be made that no spores will be produced, that is to say, asporogenous. These cells have the power of germinating after long periods of inactivity, being analogous to cells containing spores.

By "yeast ring" is meant the growth of the organism upon the inside of the flask wall, intimately adherent to the glass and not able to be washed off by moving of the fluid.

By "giant cultures" is meant a culture made upon solid media in Freudenreich flasks after this manner: with a glass rod sterilized in the flame, a drop of the fluid growth in Wort is made to fall upon the surface of the gelatine or agar and there allowed to grow.

By "bottom torula" and "top torula" is meant the characteristic of the organism of growing primarily either at the surface or at the bottom of the fluid. In "bottom growths" the top growth is secondary, and formed by the rising of cells to the surface. In "top growths" the bottom growth is secondary and is formed by the sinking of cells to the bottom of the flask.

TABLE I.

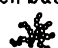
				NEOFORMANS.	SANFELICE	PLIMMER	KLEIN
MORPHOLOGY.		Spherical		+	+	+	+
MORPHOLOGY.		Oval.		(+) Occ. in old forms.	—	(+) Occ. in old forms.	—
MORPHOLOGY.		Size.		2μ to 20μ	1μ to 12μ	2μ to 10μ	2μ to 9μ
MORPHOLOGY.	Protoplasm.	Granular.		+	+	+	+
MORPHOLOGY.	Protoplasm.	Vacuoles.		+ (1 to 3)	+ (1 to 3)	+ (1 to 3)	+ (1 to 3)
MORPHOLOGY.	Protoplasm.	Oil drops.		+	+	+	+
MORPHOLOGY.	Protoplasm.	Nucleus.		+	+	+	+
MORPHOLOGY.	Protoplasm.	Mycelium		—	—	—	—
MORPHOLOGY.	Capsule	Young	Slight.	+ (very)	+	+	+ (very)
MORPHOLOGY.	Capsule	Young	Marked.	—	—	—	—
MORPHOLOGY.	Capsule	Old	Slight.	—	—	—	+
MORPHOLOGY.	Capsule	Old	Marked.	+	+ (very)	+	—
Gelatinous secretion.				+	+	—	—
TEMPERATURE.	Growth.	Maximum.		40° C.	40° C	40° C.	40° C
TEMPERATURE.	Growth.	Minimum.		5° to 7° C.	5° to 7° C.	8° to 10° C.	5° to 7° C.
TEMPERATURE.	Growth.	Optimum.		24° to 25° C.	24° to 25° C.	24° to 25° C.	24° to 25° C.
TEMPERATURE.	Life	Maximum.					
TEMPERATURE.	Life	Minimum.					
REPRODUCTION.	Moist chamber.	Open budding. 		—	—	+	+
REPRODUCTION.	Moist chamber.	Close budding. 		+	+	—	—
REPRODUCTION.	Resting cells.			+	+	+	+
REPRODUCTION.	Ascospores.	On gypsum blocks. Observations extend over six months.	45° C.	—	—	—	—
REPRODUCTION.	Ascospores.	On gypsum blocks. Observations extend over six months.	40° C.	—	—	—	—
REPRODUCTION.	Ascospores.	On gypsum blocks. Observations extend over six months.	30°-35° C.	—	—	—	—
REPRODUCTION.	Ascospores.	On gypsum blocks. Observations extend over six months.	28°-30° C.	—	—	—	—
REPRODUCTION.	Ascospores.	On gypsum blocks. Observations extend over six months.	25° C.	—	—	—	—
REPRODUCTION.	Ascospores.	On gypsum blocks. Observations extend over six months.	20°-22° C.	—	—	—	—
REPRODUCTION.	Ascospores.	On gypsum blocks. Observations extend over six months.	12°-15° C.	—	—	—	—
REPRODUCTION.	Ascospores.	On gypsum blocks. Observations extend over six months.	8°-0° C.	—	—	—	—
FERMENTATION TESTS.	Yeast water.	Lactose	15° C.	—	—	—	—
FERMENTATION TESTS.	Yeast water.	Lactose	25° C.	—	—	—	—
FERMENTATION TESTS.	Yeast water.	Lactose	35° C.	—	—	—	—
FERMENTATION TESTS.	Yeast water.	Lactose	40° C.	—	—	—	—
FERMENTATION TESTS.	Yeast water.	Dextrose	15° C.	—	—	—	—
FERMENTATION TESTS.	Yeast water.	Dextrose	25° C.	—	—	—	—
FERMENTATION TESTS.	Yeast water.	Dextrose	35° C.	—	—	—	—
FERMENTATION TESTS.	Yeast water.	Dextrose	40° C.	—	—	—	—
FERMENTATION TESTS.	Yeast water.	Saccharose	15° C.	—	—	—	—
FERMENTATION TESTS.	Yeast water.	Saccharose	25° C.	—	—	—	—
FERMENTATION TESTS.	Yeast water.	Saccharose	35° C.	—	—	—	—
FERMENTATION TESTS.	Yeast water.	Saccharose	40° C.	—	—	—	—
FERMENTATION TESTS.	Wort		15° C.	—	—	—	—
FERMENTATION TESTS.	Wort		25° C.	—	—	—	—
FERMENTATION TESTS.	Wort		35° C.	—	—	—	—
FERMENTATION TESTS.	Wort		40° C.	—	—	—	—
Facultative Anaërobic.				+	+	+	+

TABLE. II.

		NEOFORMANS.	SANFELICE	PLIMMER.	KLEIN
FILM FORMATION. WORT FREUDENREICH FLASKS.	Primary	−	+	−	+
	Secondary.	+	−	+	−
	Odor.	Of strong cheese.	Of strong stale cheese.	Of strong cheese	Of cheese.
Observations extend over six months.	45° C.	−	−	−	−
	40° C.	6 to 8 days.	5 to 6 days.	8 to 10 days.	4 to 6 days.
	30°-35° C.	3 to 4 days.	2 to 3 days	1 to 2 days.	2 to 3 days.
	28°-30° C.	72 hours.	18 to 24 hours.	48 hours.	74 hours.
	25° C.	24 hours.	10 hours.	24 hours.	18 hours.
	20°-22° C.	1 to 3 days.	1 to 8 hours.	36 hours.	36 hours.
	12°-15° C.		3 to 4 days.	4 to 5 days.	72 hours.
	8°-10° C.	3 to 4 mos.	2 weeks.	4 to 8 weeks.	1 to 2 mos.
	5°-7° C.	−	1 to 2 mos.	−	2 to 3 mos.
	−5° C.	−	−	−	−
CULTURAL APPEARANCES. TEST TUBES. LIQUID. Sediment.	Slight.	−	+	−	+
	Heavy.	+	−	+	−
Film.	Marked.	−	+	−	+
	Slight.	+	−	+	−
SOLID. Stab culture. Surface	Flat, with slight elevation.	+	+	+	+
Needle track	Stalactite form.	+	+	+	+
FREUDENREICH FLASKS. LIQUID. Film.	Marked.	−	+	−	+
	Slight.	+	−	+	−
Sediment	Fine powder	+	−	−	+
	Granular.	+	+	+	+
	Flocculent	−	+	+	−
	Clear supernatant fluid	+	+	+	+
	Cloudy on shaking.	+	+	+	+
	Yeast ring.	+	+	+	+
SOLID. Giant culture.	Flat at first.	+	+	+	+
	Elevated later.	+	+	+	+
	Beveled edges.	+	+	+	+
	Covers whole surface.	+	+	+	+
	Pasty	+	+	+	+
	Dirty yellow.	+	+	+	+
Gelatin	Liquefaction.	+	+	+	+
	Non-liquefaction.	−	−	−	−
POTATO. COLOR.	Gray with translucent areas	+	−	−	−
	Yellowish brown.	−	−	+	+
	Dark brown.	−	+	−	−
LITMUS MILK.	Acid	−	−	−	−
	Alkaline	+	+	+	+
	Neutral	−	−	−	−
BEST CONDITIONS AND MEDIA.	Acid	+	+	+	+
	Alkaline	−	−	−	−
	Neutral	−	−	−	−
	Wort	+	+	+	+
	Lactose yeast water.	−	−	−	+
	Wort agar agar.	+	+	+	+
	Bouillon	−	−	−	−
	Potato	+	+	+	+
	Wort gelatin	+	+	+	+

marked differences. As for instance, Neoformans and Plimmer, though both "bottom torulæ," are to be differentiated in that Neoformans is a "close-budding torula," while Plimmer is an "open budding torula." And the same is true of Sanfelice and Klein, both "top torulæ;" but Sanfelice is a "close budding torula," while Klein is an "open budding torula." We have here a difference which is characteristic in each case. I have found in working with these organisms that they also showed the inconstancy and variability spoken of by Hansen and other observers. At first they varied markedly in their manner of growth on the same media at different times, but finally, after months of "cultivated life" they have settled down to fairly constant characters and forms.

From the fact that they do not ferment any of the sugars and that they do not form endospores, I have concluded that these organisms are not to be classed as saccharomycetes, but as torulæ, according to the principles laid down in the first part of this paper.

When we consider that torulæ of all sorts are found in the air and earth, it is not surprising to find that different observers have found similar, if not identical, species on their culture media, even when we know that one was found in milk, one on ripe fruit, and another on the surface of media which had been planted with cancer scrapings.

I wish to acknowledge my obligations to Prof. J. Constantin of L'École Normale Supérieure, to Professor Metchnikoff of L'Institut Pasteur in Paris, to Prof. Francesco Sanfelice of the Royal University in Sardinia, to Dr. Carl Sternberg of The Rudolf-Stiftung Krankenanstalt in Vienna, to Drs. Alfred Jörgensen and Just Chr. Holm of Copenhagen for instruction and for their many kindnesses and courtesies to me.

Explanation of Signs used in Table.

+ Positive, present, or most favorable.

— Negative, not present, or least favorable.

In the experiments for ascospores and fermentation tests (Table I.) each organism was left to the temperature indi-

cated in the table for a varying time. No observation is recorded, however, unless the given flask or gypsum block had been exposed for *at least* six months; many were observed up to the ninth month.

Under film formation, Table II., the number of hours, days, weeks, or months indicated is the limit of time when the first evidences of film production were observed.

MORPHOLOGICAL AND CULTURAL DETAILS.

To make clear the description of my observations it is necessary to make a few explanatory statements.

We speak of "film" and "bottom growth," meaning pellicle and sediment. Often here there may be marked differences in the cells.

By "resting cells" is meant cells with very refractive protoplasm, oil drops and thick cell walls containing no vacuoles. When present in a given organism the inference is to be made that no spores will be produced, that is to say, asporogenous. These cells have the power of germinating after long periods of inactivity, being analogous to cells containing spores.

By "yeast ring" is meant the growth of the organism upon the inside of the flask wall, intimately adherent to the glass and not able to be washed off by moving of the fluid.

By "giant cultures" is meant a culture made upon solid media in Freudenreich flasks after this manner: with a glass rod sterilized in the flame, a drop of the fluid growth in Wort is made to fall upon the surface of the gelatine or agar and there allowed to grow.

By "bottom torula" and "top torula" is meant the characteristic of the organism of growing primarily either at the surface or at the bottom of the fluid. In "bottom growths" the top growth is secondary, and formed by the rising of cells to the surface. In "top growths" the bottom growth is secondary and is formed by the sinking of cells to the bottom of the flask.

T. Neoformans (Sanfelice).— The Young Growth after twenty-four hours in Wort at 24° C.

Cells are round, colorless, size from four to eighteen mikrons in diameter. In this early form the capsule is very delicate, occasionally showing a double layer. There are occasional vacuoles with considerable numbers of oil drops in the protoplasm, which is either finely granular or homogeneous. Budding is often evident mostly in cells of the bottom growth. This description holds true for both the bottom growth and the film.

Older forms (four to five months) on Worth agar-agar.

Cells mostly round or oval, many very large, round, oval, and distorted forms. Size varies from two to twenty-two mikrons, and even larger forms representing incomplete separation and independent growth of a daughter cell. Large vacuoles are seen containing the so-called nucleus of the vacuole. Vacuoles number from one to three in a single cell. Large and small oil drops from one to six in a cell are seen, these cells having no vacuoles. The protoplasm is either finely or very coarsely granular. Each cell seems to have a well-marked capsule, often very thick. There are many "resting cells." Budding but very rarely seen. (Plate XXII., Figs. b and c.)

Old film cells (four to five weeks): Cells are large, colorless, and round, with slight amount of budding.

Old bottom growth: Size two to twenty mikrons in diameter. Cells are all round with capsules very well marked. Large oil drops and great amount of budding with some dumb-bell forms. Cells slightly yellowish in color.

Ring: Large round cells, more or less yellowish to brown in color. Size six to twenty mikrons. Very granular protoplasm.

T. Sanfelice.— The Young Growth after twenty-four hours in Wort at 24° C.

Cells of film are round, colorless, size from one to eight mikrons. The great majority are in the process of budding.

The capsules are extremely delicate, some cells with absolutely no capsules, these being the smallest. Oblong and misshapen cells are seen, being in process of budding. The complete cell is always round. The protoplasm is homogeneous in almost all the cells. Occasionally it is granular, containing oil drops. In the bottom growth cells are round, size three to twelve mikrons in diameter. All the cells have a highly refracting ring (capsule). Few evidences of budding. Protoplasm is very granular with some oil globules and vacuoles. All the large cells, ten to twelve mikrons, have a quite homogeneous protoplasm with tiny oil drops, and all the small cells have a very granular, highly refracting protoplasm. There are vacuoles occasionally in the large cells. (Plate XXI., Fig. a.)

Older forms (four to five months) on Wort agar-agar.

All the cells are round and markedly different in appearance. Some have a capsule one to one and five-tenths mikrons in thickness. Many have an outer layer about the capsule of great thickness. This is evidently a part of the gelatinous network. This outer covering is often found to have the appearance of being torn off in handling. Occasionally the whole covering is seen free in horse-shoe form, the cell itself escaping from it. No distortion. Very many resting cells. Large amounts of free oil drops in active brownian motion. No vacuoles. Large granules and fat globules. General appearance of the cut surface of a pomegranate. Occasional budding. (Plate XXI., Fig. b, and Plate XXII., Fig. a.)

Old film cells (four to five weeks): Cells round, large, in process of budding. Capsules well marked; no gelatinous covering.

Old bottom growth: Cells are round, yellowish, large. Protoplasm granular with oil drops. No gelatinous covering. Large capsules, with occasional vacuoles. Almost no budding seen.

Ring: Large round cells, more or less yellowish brown in color, and very granular.

T. Plimmer.— The Young Growth after twenty-four hours in Wort at 24° C.

Cells are round, colorless, size from four to eight mikrons, almost universally no capsules. Occasionally seen, however, in the large cells. Vacuoles and considerable numbers of oil drops in the protoplasm, which is very granular. Budding is well marked. In the "bottom growth" the cells are all round, size from two to ten mikrons. Capsules well marked in large cells, absent in the small cells. Protoplasm homogeneous or finely granular, with occasional vacuole and fat globule. Slight amount of budding. (Plate XXI., Fig. c.)

Older forms (four to five months) on Wort agar-agar.

Cells mostly round or oval, few distorted forms. Size varies from two to sixteen mikrons. Large vacuoles are seen, one to three in a single cell. Protoplasm either finely or coarsely granular. Cells all have capsules more or less well marked. There are many resting cells, and cells in chains of three, with a characteristic interposition of a small cell between two large cells. Budding occasionally seen. (Plate XXI., Fig. d.)

Old film cells (four to five weeks): Cells are large, colorless, some evidences of budding and chains of three. (Plate XXI., Fig. d.)

Old bottom growth: Cells are all round, with capsules well marked. Cells slightly yellowish in color, some in process of budding.

Ring: Large round cells more or less yellowish in color, with very granular protoplasm.

T. Klein.— The Young Growth after twenty-four hours in Wort at 24° C.

Cells of film are round or slightly oval, colorless, size from two to eight mikrons. Great majority in process of budding. No capsules evident, even in large cells. No misshapen cells. The protoplasm is finely or coarsely granular, with few oil drops and large vacuoles. Budding is marked. In the bottom growth the cells are round, colorless, size two to nine

mikrons. The capsule here is occasionally present. Protoplasm is very granular in all the cells, large and small, with large numbers of fat globules and few vacuoles. Budding only occasionally seen. (Plate XXI., Fig. e.)

Older forms (four to five months) on Wort agar-agar.

All the cells are round and very coarsely granular, with some cells containing one large vacuole, others with many fat globules. Each cell has a fairly well-marked capsule. There are many resting cells. Budding rarely seen. (Plate XXI., Fig. f.)

Old film cells (four to five weeks): Cells are of medium size. Many in process of budding. Capsules well marked in all cells. No gelatinous covering seen.

Old bottom growth: Cells are all round, protoplasm very granular with oil drops. Cells yellowish in color, capsules well marked. Very slight amount of budding.

Ring: Medium size round cells, more or less yellowish to brown in color. Very coarsely granular protoplasm.

Moist Chamber, in Wort, at 25° C.

T. Neoformans. — After three and one-half hours, budding first began. After six hours, budding well marked. After twelve hours, growth, and after twenty-four hours, growth characterized by *close* grouping of cells in clumps. An occasional strand of six to eight cells with few side buds. Cells are round, growth in all planes. An occasional oblong bud. *Close budding.*

T. Sanfelice. — After two and one-half hours, budding began. After five hours, budding marked. After twelve hours, growth, and after eighteen hours, growth characterized by *close* grouping of cells. All cells round. Growth in all planes. *Close budding.*

T. Plimmer. — After four hours, budding began. After eight hours, budding well marked. After eighteen hours, growth characterized by round and oval cells. Budding more or less loose or *open.* In the small colonies the growth is in one plane and marked by straight lines of cells. The

arrangement often is the interplacement of a small cell between two large ones. *Open budding.* (Plate XXI., Fig. c.)

T. Klein. — After four hours, budding began. After seven and one-half hours, budding well marked. After eighteen hours, growth characterized by round and oval cells. Budding more or less open in small colonies with chains running out, with side budding and branching effects. Budding is in one plane *Open budding.*

Wort. Freudenreich Flasks at 25° C. (Plate XXIV.)

T. Neoformans. — Fluid remains clear. Film first formed after twenty-four hours and is very slight indeed. After forty-eight hours still very delicate, and not until after ten days to two weeks is the film well marked. Its character is delicate, somewhat granular or cheesy. This characteristic is not constant. It is occasionally veil-like.

Bottom growth: First formed after ten hours. Very slight. Becomes very heavy in from forty-eight to seventy-two hours. Time variable. The character of bottom growth varies. It may be granular or powder-like, occasionally flocculent. The bottom growth is always more marked than the top, hence the conclusion that this is a "*bottom torula.*"

The color of the fluid becomes very much lighter brown as the growth progresses, and by agitation of the flask becomes a dirty yellow, opaque, and cloudy. The ring, however, remains after shaking.

Ring: Slight, but evidence after twenty-four hours. Later becomes very well marked. Character is nodular, slightly yellowish in color and translucent.

T. Sanfelice. — Fluid remains clear. Film first formed after ten hours; very delicate, becoming very heavy and membranous later. The character is much the same as that of Neoformans, and is variable in the same manner.

Bottom growth: First formed after twenty-four hours, always less than the film, until after two or three weeks the film falls *en masse* to the bottom. The reformation of the film takes place, but very slowly, and is always very delicate. The bottom growth then is very heavy, cheesy, flaky, even membranous in character. This, then, is a "*top torula.*"

Ring: Evident after eighteen hours. Later very heavy, uniform, yellowish, and opaque.

Fluid remains clear, but loses much of its brown color, becoming lighter brown to yellow as the growth progresses. By agitation of the flask there is the same result as in the Neoformans. The ring remains constant.

T. Plimmer. — Fluid remains clear. Film first formed after twenty-four hours; very delicate, and remains more or less delicate always. Its character is veil or scum-like, and is more or less constantly so. It does not fall to the bottom.

Bottom growth: First formed after eighteen hours and becomes rather heavy later. Character more or less constantly cheesy or coarsely granular. The bottom growth is evidently primary and hence this is a "*bottom torula.*"

Ring: Evident after twenty-four hours and is well marked usually, although not always. It is heavy and opaque. Occasionally, however, it may be very delicate.

The fluid pales out with time and presents the same characters as in the others.

T. Klein. — Fluid remains clear. Film is first formed after 18 hours and is at first delicate, and although remaining veil-like always, it becomes later more and more membranous.

Bottom growth: First formed after twenty-four hours and is more or less flocculent or granular. It becomes marked later in varying lengths of time. This growth is evidently secondary to the top growth, and hence this is a "*top torula.*" Often are there showers of cells to be observed falling more or less constantly from the under surface of the film to the bottom of the flask.

Ring: Evident first after forty-eight hours, and only after four days is it at all well marked.

The fluid pales out as in the other cases and, although remaining clear, when very slightly agitated we get marked showers of cells. Active agitation gives the same result as in the three former organisms.

In the case of all four organisms there was no trace of fermentation of the maltose.

The odor in each case is that of more or less stale cheese, resembling also the peculiar odor of bread yeast.

Film-formation Experiments.

In Freudenreich Flasks — Wort.

T. Neoformans.

At 45° C. no film formation occurred.

At 40° C. feebly developed specks are seen after 6–8 days.

At 30–35° C. feebly developed specks are seen after 3–4 days.

At 28–30° C. feebly developed specks are seen after 72 hours.

At 25° C. feebly developed specks are seen after 24 hours.

At 20–22° C. feebly developed specks are seen after 1–3 days.

At 15–12° C. feebly developed specks are seen after 1–2 months.

At 8–10° C. feebly developed specks are seen after 3–4 months.

At 5–7° C. no film formation occurred.

Optimum temperature, 25° C. Maximum, 40° C. Minimum, 5–7° C.

T. Sanfelice.

At 45° C. no film formation occurred.

At 40° C. feebly developed specks are seen after 5–6 days.

At 30–35° C. feebly developed specks are seen after 2–3 days.

At 28–30° C. feebly developed specks are seen after 18–24 hours.

At 25° C. feebly developed specks are seen after 10 hours.

At 20–22° C. feebly developed specks are seen after 18 hours.

At 15–12° C. feebly developed specks are seen after 3–4 hours.

At 8–10° C. feebly developed specks are seen after 2 weeks.

At 5–7° C. feebly developed specks are seen after 1–2 months.

At 5° C. (on ice) no film formation occurred.

Optimum temperature, 25° C. Maximum, 40° C. Minimum, 5–7° C.

T. Plimmer.

At 45° C. no film formation occurred.

At 40° C. feebly developed specks are seen after 8–10 days.

At 30–35° C. feebly developed specks are seen after 1–2 days.

At 28–30° C. feebly developed specks are seen after 48 hours.

At 25° C. feebly developed specks are seen after 24 hours.

At 20–22° C. feebly developed specks are seen after 36 hours.

At 15–12° C. feebly developed specks are seen after 4–5 days.

At 8–10° C. feebly developed specks are seen after 4–8 weeks.

At 5–7° C. no film formation occurred.

Optimum temperature, 25° C. Maximum, 40° C. Minimum, 8–10° C.

T. Klein.

At 45° C. no film formation occurred.

At 40° C. feebly developed specks are seen after 4–6 days.

At 30–35° C. feebly developed specks are seen after 2–3 days.

At 28–30° C. feebly developed specks are seen after 17 hours.

At 25° C. feebly developed specks are seen after 8 hours.

At 20–22° C. feebly developed specks are seen after 36 hours.

At 15–12° C. feebly developed specks are seen after 72 hours.

At 8–10° C. feebly developed specks are seen after 1–2 months.

At 5–7° C. feebly developed specks are seen after 2–3 months.

At 5° C. (on ice) no film formation occurred.

Optimum temperature, 25° C. Maximum, 40° C. Minimum, 5–7° C.

T. Neoformans. — Wort gelatine plates. After three or four days the colonies show characteristics. Those upon the surface which are confluent are flat, somewhat elevated at the edges, with slightly depressed centres. Surface is dull and smooth, resembling wax. The color a yellowish white. Individual colonies remain often about the size of a large pin head and even after weeks do not increase in size. These are heaped up, markedly elevated, even to the shape of a hemisphere with a dull sheen much like that of a non-lustrous pearl. There is very slight development beyond a pin-point size in the deep colonies.

T. Sanfelice. — The appearance and time necessary for growth all correspond with that of T. Neoformans. There is not the least difference observable between these two on gelatine plates.

T. Plimmer. — Here again there is but very little variation from the appearances of T. Neoformans. The confluent colonies are, perhaps, somewhat smoother, the centres being not so depressed, and again the edges are but very slightly if at all elevated. The individual colonies, which remain about the size of a large pin-head, are, however, as markedly elevated as in Neoformans and the deep colonies here also develop but slightly beyond a pin-point size.

T. Klein. — A difference in the plates is occasionally seen in the case of this organism, which, however, is not constant. The centers of the large, confluent, flat, superficial, nastur-

tium leaf shaped colonies are occasionally seen to be markedly elevated and sharply defined, not unlike a pin being pushed into the leaf's center up to the head. Just surrounding this prominent elevated center is usually a translucent area in the colonies, and again outside of this area the waxy, yellowish-white, flat characteristic appearance. The small surface and deep colonies are in every particular similar to Neoformans.

In the case of all four organisms, liquefaction of the gelatine is very slow, occurring only after six to ten days, — often no liquefaction, the gelatine becoming dried up before it takes place.

GIANT CULTURES.

T. Neoformans on Wort Agar-agar in Freudenreich. Flasks at a Temperature of 25° C.

After twenty-four hours a very slight transparent colorless layer, with a rather rough surface, slightly glistening. After forty-eight hours the growth is whitish, translucent, and more elevated, and later this elevation becomes very marked. At the end of a week the growth is entirely opaque, yellowish-white, and very markedly elevated with bevelled edges. In time, three to five weeks, the whole surface of the media is covered, the growth then extending to the walls of the flask and is uniformly yellowish-brown, and pasty in consistency. This growth may reach a thickness of a quarter to half an inch.

T. Sanfelice. — After twenty-four hours the same condition as in T. Neoformans and only after a lapse of a week or more are there any differences to be observed. The bevelling is at a sharper angle than in any of the four organisms, and the yellow color is more uniformly yellow, the whole growth being more compact, more even, and clean cut than any of the others. The end result, when the growth reaches the walls of the flask, is not different from Neoformans, excepting in a slightly more decided yellowish color.

T. Plimmer. — The first stages are much the same as in the above two organisms. Later we have slightly characteristic

differences. There is a decided ribbing of the growth, a radiation of lines from the center, and a more or less constant effect of concentric circles, giving the appearance of the inside of a highly developed cell. The bevelling is at a much more obtuse angle and the extreme limit of the growth is pure white, while the center is uniformly yellow. The end result, however, is in no way different from T. Neoformans.

T. Klein. — Here also we have the same beginning. Later the growth shows much the same condition as T. Neoformans, excepting that the bevelling seems to be at a somewhat more obtuse angle. The center of the growth is uniformly yellow, while the edges are a mixed yellow and white. There is also an approach to the shell-like resemblance. The end result varies in no way from Sanfelice and Neoformans.

Giant Cultures on Wort Gelatine in Freudenreich Flasks.

T. Neoformans. — After twenty-four hours an almost invisible growth, perfectly transparent, with all the characteristics of the agar-agar growth. After seven to eight days there is beginning liquefaction. This continues steadily, until at the end of five or six weeks there is complete liquefaction of the whole media. The color change takes place as in Wort, and there is a sediment or bottom growth with film formation, with, however, but slight tendency to ring formation.

T. Sanfelice. — After twenty-four hours the same characteristic growth begins as on agar-agar, and as in the case of Neoformans, progresses with no differences, until beginning liquefaction in from seven to eight days, when the whole bevelled mass begins to sink into the liquefied gelatine, and in about five or six weeks there is complete liquefaction with bottom and top growths and slight ring formation.

T. Plimmer. — There is no essential difference from the above two descriptions, the growth is as on agar-agar, and the liquefaction begins after seven or eight days, being complete in five or six weeks, with the same bottom and top growths and slight ring formation.

T. Klein. — Here also is the same elevated bevelled growth as on agar-agar. Beginning liquefaction after seven or eight

days with sinking of the mass. Complete liquefaction in five to six weeks. T. Klein shows on the liquefied gelatine a notable characteristic, that is, a very marked, opaque, thick film with a marked, slightly yellowish ring. This is the only case in which I have seen a marked ring formation with a heavy film as well. There is also the characteristic bottom growth.

The growth of these organisms in

1. Plain yeast water,
2. Saccharose yeast water,
3. Lactose yeast water, and
4. Dextrose yeast water, shows nothing peculiar.

The films are fairly well marked, and in the case of T. Klein the film and growth in general are especially well marked in lactose yeast water. (This is interesting, for we know this organism was found by Klein in milk.)

The object of growing these organisms in these fluids was primarily for the fermentation tests of the different sugars. At temperatures varying from 40° C. to 15° C., the observations extending over a period of from six to nine months, no fermentation was observed.

There is no ring formation in these solutions.

In the case of all four organisms in all four different media, there was no change in the reaction.

Observations in saccharose solution, ten per cent, or saccharose yeast water, after Fehling's solution had been added, show no Invertin, even under the microscope within the cell itself.

There is, therefore, no fermentation of maltose, saccharose, dextrose, or lactose by any of these four organisms.

EXPERIMENTS FOR ASCOSPORE FORMATION.

All four organisms were exposed upon Gypsum blocks to temperatures ranging from about 0° C. to 45° C., for a period of from six to nine months, and observations were made beginning after twenty-four hours.

All organisms showed uniformly no trace of spore formation.

This fact is of great importance for the classification of these organisms, for, as has already been pointed out, this characteristic alone rules them out of the saccharomycetes group.

On Potato in Test Tubes, 24° C. (Plate XXIII.)

All four organisms after twenty-four hours showed a slightly elevated, pure white, moist, more or less slimy, glistening layer. This growth after forty-eight hours begins to take on a yellowish-gray color which later in each case develops a characteristic deep yellow or brown, varying in the case of each organism.

The growth itself develops rapidly with markedly elevated edges and somewhat characteristic surface appearances.

T. Neoformans presents a gray or drab color. The edges and surface, while being slightly irregular, are much smoother and straighter than in the case of the other organisms. Here and there throughout the growth are translucent areas which are composed of fat.

T. Sanfelice presents the darkest brown color, with very irregular outline and a somewhat glistening and corrugated surface.

T. Plimmer presents a yellowish-brown color, the surface being markedly convoluted and the outline very irregular.

T. Klein presents a slightly more yellowish tint, its surface being the most markedly convoluted and the edges the most irregular.

At 37° C.

The same characteristics are developed at this temperature, excepting only the growth is delayed from five to six days.

Stab Cultures in Wort Agar-Agar in Freudenreich Flasks and in Test Tubes.

The surface growth in each case is similar to that of the giant cultures on the same media, but is much less elevated.

Along the needle track there is a spiked growth somewhat of the "inverted pine tree" character, resembling, however, more a stalactite formation, the spikelets being all rounded or blunt.

The needle track differs slightly in certain of the organisms.

T. Neoformans shows the heaviest spikelets as well as the longest, extending farthest into the surrounding media.

T. Sanfelice, T. Plimmer, and T. Klein show very short spikelets, being all closely confined to the area just at the edge of the needle tract.

Stab cultures in Wort gelatine, in yeast-water agar-agar, and yeast-water gelatine all show these same peculiarities. In the case of the gelatine media liquefaction at the end of from six to eight days masks the picture.

Blood Serum in Test Tubes, 25° C.

There is no growth after thirty-six hours. After forty-eight hours there is a beginning growth which is similar for all four organisms, a milk white, more or less elevated, slightly slimy, glistening layer which gradually takes on a yellow color. The growth is very meagre. Even after months there is very little increase in the amount which is originally seen after forty-eight to seventy-two hours. As the media dries out the growth becomes chalky, whitish-yellow, like daubs of white lead paint.

At 37° C.

There was uniformly no growth after four days. After one hundred and eight hours T. Klein and T. Sanfelice began first to show evidences of growth, and after six days Neoformans and T. Plimmer first showed a growth. In all four cases the growth was very slight, whitish, and quite flat. After two weeks in the incubator there was no further development, but after removal from the incubator and at the room temperature there was a slight increase in the amount of the growth which was similar to that at the temperature of 25° C. The drying of the media also produced the white lead like appearance.

Litmus Milk in Test Tubes, 25° C.

No action after twenty-four hours. After ninety-six hours still no change. Indeed, there never was any marked change. After two months all four tubes were still blue in color, consistency could not well be called coagulation in any case, but in the case of T. Klein the growth was so profuse that the liquid was considerably thickened, and simulated coagulation to some extent. The other three showed this same thickening of the media to a much less extent.

At 37° C.

The same results were obtained, that is, no change in reaction. After about two months Torula Klein showed what I call "false coagulation," as described above, and the other three showed a slight amount of thickening of fluid also.

I explain this by evaporation, and also by the growth of the organisms which by its very presence, perhaps, helps in causing this condition. It may, however, be stated that in no case was there a good growth until after four to six weeks, and in no case were any characteristics shown. There were no film formations.

Glucose Bouillon in Test Tubes, 25° C.

After twenty-four hours no visible growth. After forty-eight hours a beginning growth. In the case of T. Klein the film was well marked and in T. Sanfelice the film was fairly well defined, while T. Neoformans and T. Plimmer showed a very feeble bottom growth. Only after seven days are the growths well-defined. The fluid remains uniformly clear. T. Plimmer shows the most feeble growth, while T. Klein and T. Sanfelice show the best growth. Fairly heavy films, with more or less tendency to growth on to the side of the tube simulating a ring. This is not a true ring, however, as it is possible to wash it off by tipping the tube. Agitation of the tube causes cloudiness in the fluid.

At 37° C.

There was no growth in any of the tubes after forty-eight hours. After ninety-six hours T. Neoformans and T. Plim-

mer show no film, but a slight bottom growth or sediment, while T. Klein and T. Sanfelice show a beginning film and no sediment. Fluid remained clear; cloudy only after shaking.

The character of the growth is not different at either temperature from that on Wort, the film being white, later becoming yellowish-white, the sediment being white to grayish-white. There was no fermentation and no change in reaction.

In a ten per cent saccharose bouillon, to which has been added one-half per cent tartaric acid (a medium recommended by Sanfelice), the growth of all four organisms in no way differs from that in glucose bouillon.

REFERENCES.

1. Adamatz, L. Saccharomyces lactis, eine neue Milchzucher vergärende Hefeart. Centr. f. Bakt., v., 1889.

2. Aubry, L. Ein Beitrag zur Klärung und Richtigstellung der Ansichten über reine Hefe. Zeitschr. f. d. ges. Brauwesen, 1885.

3. De Bary. Comparative morphology and biology of the Fungi Mycetozoa and Bacteria. Oxford, 1887.

4. Bay, J. Chr. Is the red torula a genuine saccharomyces? Centr. f. Bakt. u. Par., 2 abt., ii., 1896.

5. Boullanger, E. Action des levures de biere sur la lait. Ann. de L'Institut Pasteur, xi., 1897.

6. Busse. Über saccharomyces hominis. Arch. f. path. Anat. u. Phys., cxl., 1895, and Centr. f. Bakt., xvi., 1895.

7. Corselli e Frisco. Blastomycete patogeno nell' uomo. Ann. d'Igene speriment, v., 1895.

8. Casagrandi. Il saccharomyces ruber. Ibid., iv., p. 535, 1897.

" Sulla diagnosi differentiale dei blastomyceti. Ibid., iii., p. 318, 1889.

9. Duclaux. Sur la conservation des levures. Ann. de L'Institut Pasteur, iii., 1889.

Duclaux. Notes sur la formation des spores dans les levures. Ibid., iii., 1896.

10. Fermi. Studio biologico sul blastomyceti. Il Policlinico, 1896.

11. Flügge, G. Die Mikroorganismen. Leipsig, 1896.

12. Gilchrist, Casper and Stokes. A case of pseudo-lupus vulgaris caused by a blastomyces. Centr. f. Allg. Path. u. path. Anat., March, 1899.

13. Hansen, E. Chr. Praxis der Garungsindustrie. Munich and Leipsig, 1895.

Hansen, E. Chr. Compte-Rendu des travaux du Lab. de Carlsberg. Copenhagen, from 1879 to 1901.

14. Holm, J. Chr. Über die Autbewahrung der Hefe in Saccharoselösung. Centr. f. Bakt. u. Par., 2 abt., ii., 1896.

15. Holm and Poulsen. Jusqu'à quelle limite peut-on constater une infection de levures sauvages. Compte-Rendu des trav. du Lab. de Carlsberg, ii., 4, 1886, and ii., 5, 1888.

16. Janssens, Fr. A. Beiträge zu der Frage über den Kern der Hefezelle. Centr. f. Bakt. u. Par., xiii., 1893.

17. Jörgensen, Alfred. Micro-organisms and Fermentation. London, 1900.

18. Klöcker, Albert. Die Gärungsorganismen in der Teorie und Praxis der Alkoholgärungsgewerbe. Stuttgart, 1900.

19. Linder, P. Die Askosporen und ihre Beziehungen zur Konstanz der Hefenrassen. Wochenschr. f. Brau., 1887.

Linder, P. Nachweis von Mikroorganismen in der Luft von Gärungsbetrieben. Ibid., 1887.

Linder, P. Das Wachstum der Hefen auf festen Nahrböden. Ibid., 1895.

20. Metchnikoff. Notes au sujet du mémoire de M. Soudakewitch. Ann. de L'Institut Pasteur, 1892.

21. Maffucci e Sirleo. Osservazione ed esperimenti interno ad un blastomycete patogeno. Il Policlinico, v., May, 1895.

22. Migula, W. System der Bakterien. 1897 and 1900.

23. Miguel. Des organismes vivantes de l'atmosphère. Paris, 1893.

24. Naegeli. Die niederen Pilze in ihren Beziehungen zu den Infektionskrankheiten. Munich, 1877.

25. Pasteur, L. Études sur le vin. 1872.
" Études sur le vinaigre. 1868.
" Études sur la biere. 1876.

26. Rabinowitsch, L. Untersuchungen über pathogene Hefearten. Zeitschr. f. Hyg., Bd. xxi., 1895.

27. Rossi-Doria. I blastomyceti nel sarcoma puerperale infettante. Il Policlinico, Feb., 1893.

28. Sanfelice, F. Contributo alla morphologia e biologia dei blastomyceti che si svillupano nei succhi di alcuni frutti. Ann. d'Igene sperimentale, 1894.

Sanfelice, F. Über eine für Tiere pathogene Sprosspilze, etc. Centr. f. Bakt. u. Par., p. 113, 1895.

Sanfelice, F. Über einen neuen pathogenen Blastomyceten. Ibid., xviii., p. 521, 1895.

Sanfelice, F. De l'action pathogène des blastomycetes. Centr. f. Bakt., xvii., 1895.

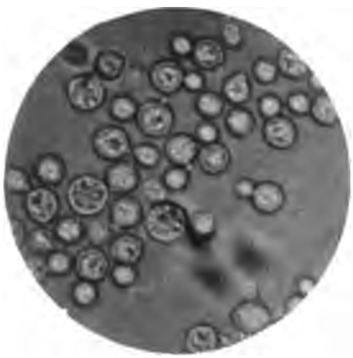
29. Tieghem, Ph. van, et Le Monnier. Recherches sur les Mucorinées. Ann. des sc. nat. Bot. V., xvii., 1873.

30. Will, H. Wie wird reine Hefe gezüchtet? Zeitschr. f. d. ges. Brau., 1885.

Will, H. Die Methoden, etc. Centr. f. Bakt. u. Par., 2 abt., ii., 1896.

Will, H. Bemerkungen zu Mitteilung von Casagrandi: über die Morphologie der blastomyceten. Ibid., 1898.

31. Wilhelmi. Beiträge zur Kenntniss des Saccharomyces guttulatus. Centr. f. Bakt. u. Par., May, 1898.



A

B

C

D

E

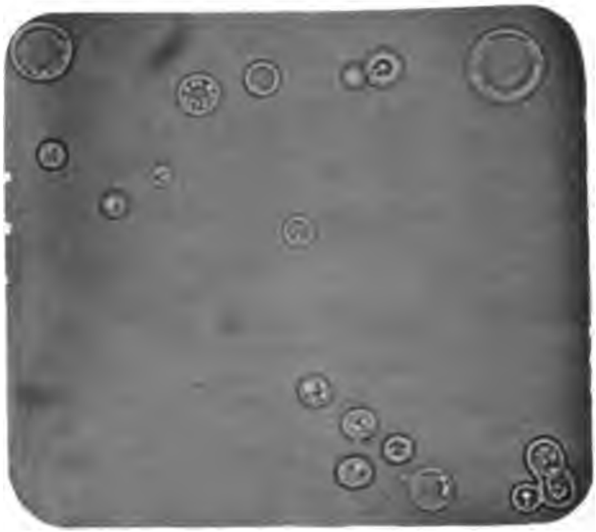
F

Weis. **Pathogenic Torulae.**

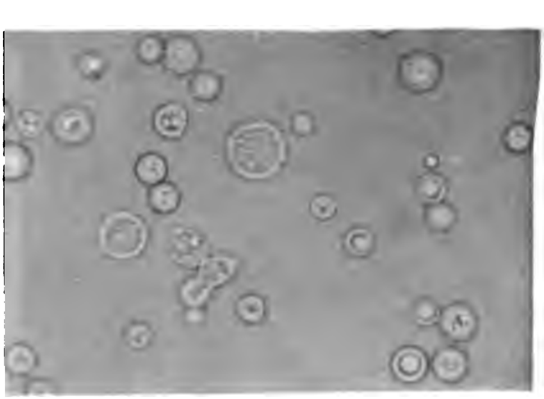
A

D

E



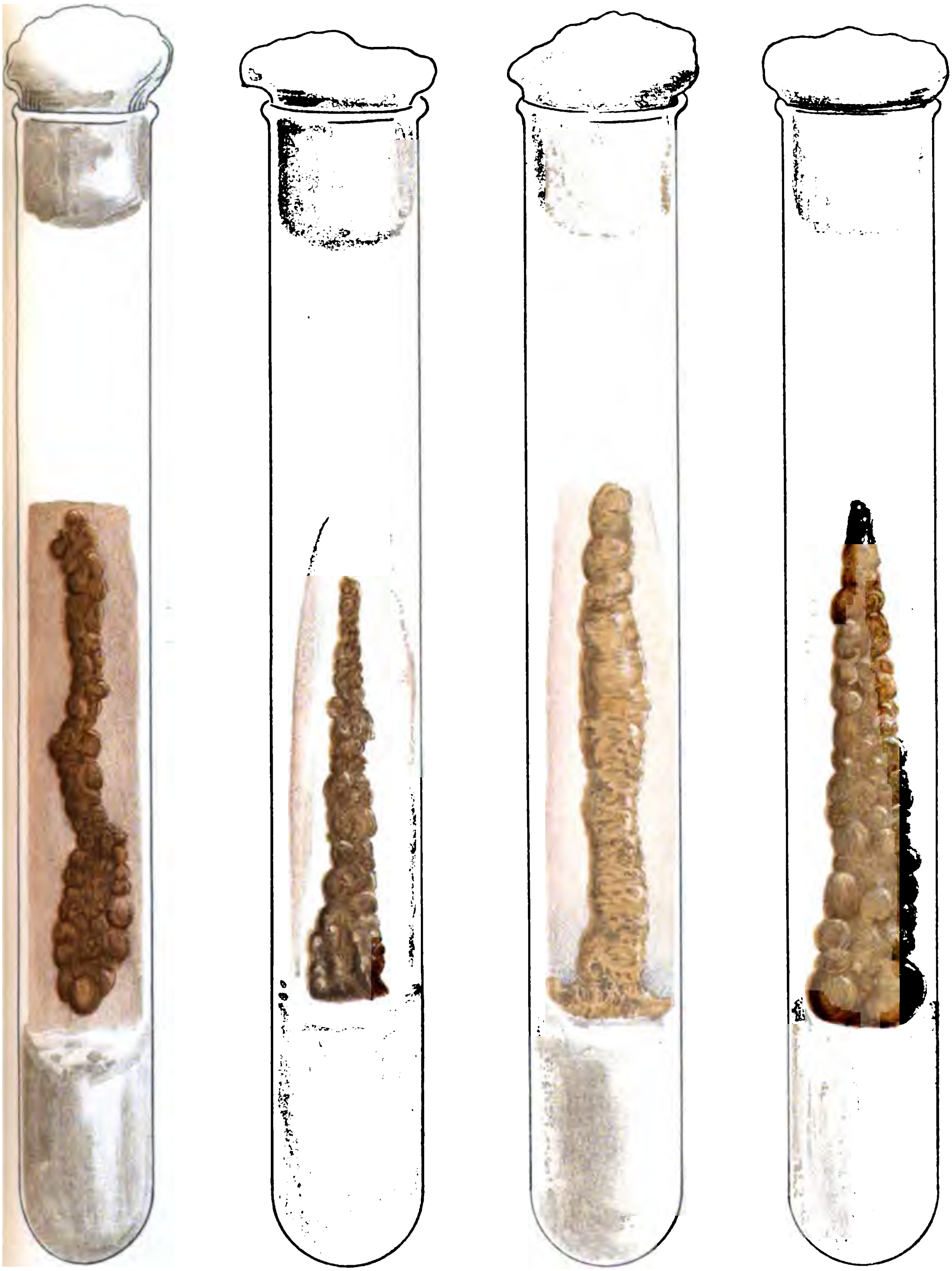
B



C

Weis. Pathogenic Torulae.

Heliotype Co, Boston



A B C D

A B C D

Weis Pathogenic Torulae

EXPLANATION OF PLATES.

Plate XXI.

a. Young forms of T. Sanfelice, showing in some cases a capsule, also tendency to "close budding."

b. Old forms of T. Sanfelice, showing thick capsules, horse-shoe form of broken-off gelatinous covering, and granular protoplasm with oil drops.

c. Young forms of T. Plimmer (moist chamber growth), showing interplacement of small cell between two larger ones, also "open budding." These cells may well stand for T. Neoformans, as there is no difference in individual young cells.

d. Old forms, T. Plimmer, showing capsules and chains of three.

e. Young forms of T. Klein, showing round and slightly oval cells without capsules.

f. Old forms, T. Klein, showing coarse granular protoplasm, pomegranate effect — large oil drops and vacuoles.

Plate XXII.

a. Old forms of T. Sanfelice, showing variety in size and conformity. Almost every form assumed by this organism is shown in this picture.

b and *c.* Old forms of T. Neoformans, showing very large and bizarre forms as well, with smaller round cells.

d and *e.* Examples of true endospore formation. (*d.*) Sacch. Ludwigii (Hansen). (*e.*) Sacch. Cerevisiæ I. (Hansen).

Plate XXIII.

a. Growth upon potato at 24° C. to show color production and appearances: Of T. Klein, color and conformation of growth well shown.

b. Of T. Sanfelice.

c. Of T. Neoformans; clear translucent areas in growth which are accumulations of fat (degeneration) are well shown.

d. Of T. Plimmer.

Plate XXIV.

Growth in Freudenreich flasks in Wort at temperature 24° C. To show "yeast ring," "films," and "bottom growths."

a. Torula Sanfelice. Heavy ring. Well represented.

b. T. Neoformans. The nodular character of ring shown.

c. T. Plimmer.

d. T. Klein. Veil-like character of film well shown.

[Photomicrographs by Mr. L. S. Brown. Colored drawings by Miss Florence Byrnes.]

THE RELATION OF BLASTOMYCETES TO CANCER.

EDWARD H. NICHOLS, A.M., M.D.
(Demonstrator of Surgical Pathology, the Harvard Medical School; Assistant Visiting Surgeon, the Boston City Hospital.)

(From the Sears Pathological Laboratory of the Harvard Medical School.)

Many men, on inconclusive evidence, have during recent years assumed that carcinomata were produced by living parasites. Since Virchow's[1] time it has been well known that often in the epithelial cells of carcinomata were found certain peculiar bodies, whose presence was not easily explained. During the past fifteen years, many men, attracted by the hope of finding a cause for the overgrowth of the epithelial cells, have endeavored to prove that these peculiar cell inclusions were parasites. Some men have called these inclusions protozoa; others have claimed that they were blastomycetes.

The idea that these cell inclusions are blastomycetes practically dates from an article by Busse[2] published in 1894. In what was essentially a preliminary report he described certain bodies which were found in the fresh tissue taken from what, clinically, was considered a chronic subperiosteal inflammation, or a "soft sarcoma" of the tibia of a woman. The tumor had developed slowly. The microscopic examination of the tissue showed young connective tissue, and numerous giant cells, between and in which were numerous small, circular refractile bodies, with a double contour. Busse put small bits of this tissue into the tibia of a rabbit, and tied a tourniquet above the point of inoculation. The leg became gangrenous, was amputated, and examination showed that the parasites had multiplied. Busse also inoculated some of the tissue beneath the periosteum of a dog and closed the wound. The wound opened in three days and discharged pus, and in the pus were many of the bodies, and giant cells were seen. Some of the bodies were in-

cluded in large phagocytic cells. The wound healed in twelve days, and the bone thickened. He put some of the pus into the belly of a rabbit, and produced a fibrinous peritonitis and enlargement of the mesenteric lymph nodes. The lymph nodes and the inflammatory areas contained the bodies. Busse also inoculated a dog with pus from the peritoneal cavity of the rabbit and produced an abscess which contained the bodies. Busse stated that ordinary pyogenic organisms were present in the abscess, and granted that the bodies did not produce all of the reaction. Nor at that time did he make any definite statement regarding the pathological process.

Busse cultivated the organisms upon ordinary media, getting the best growth upon potato. He also grew them on jelly and produced carbonic acid; hence he decided that the organisms probably were yeasts.

In a second paper,[3] published some months later, Busse gave a more complete clinical history of his case, the result of the autopsy, a description of the histology of the various organs affected, a minute description of the organism, and the results of the inoculation of animals. The patient was a woman, who in her youth had been operated on for tuberculous glands of the neck. A short time after the birth of her third child pain and swelling appeared over the left tibia. Soon afterwards the left knee swelled, but finally the effusion in the knee disappeared, while the swelling of the tibia increased. The leg swelling was incised, but the wound did not heal well and sinuses persisted. Ulcers appeared upon the face, which did not tend to heal. Later there came areas of inflammation in other bones, including the ulna, and these areas opened and discharged material like that from the tibial wound. The woman died after nine months, and at autopsy there were various ulcers of the skin, bone lesions, and general enlargement of the lymph nodes, a nodule the size of a walnut in the spleen, with central softening, a nodule of softening in one kidney, and an abscess of the lung, but no foci in the liver. Busse specifically stated that the lesions were *inflammatory and not sarcomata.*

In pus from the bone lesions were typical yeasts. Similar yeasts were found in the new nodules, in the pleura, and in macroscopically unchanged tissue of the lungs, in the kidney abscess, and in areas in the spleen. That is, the process was a "general infection, differing from pyæmia by the presence of giant cells and the peculiar parasitic bodies."

Busse got a pure culture of these parasites. He found that they grew best on potato, at room temperature, were durable for months, and produced a fermentation in sugar media.

Busse inoculated animals with these pure cultures. He produced nodules of suppuration, at the point of inoculation, which diminished after a few weeks or months. In the nodules was granulation tissue in which were yeasts; *i.e.*, the nodules were *inflammatory thickenings*. Yeasts either were in phagocytic cells or in spaces between cells and usually were surrounded by a gelatinous capsule which arose either from the yeast or from the tissues, Busse was unable to say which. In mice he produced a septicæmia.

In a third article,[4] in 1896, Busse published the results of inoculation experiments with the blastomyces from his patient, in greater detail. He used white mice in his experiments. He found that the animals died in from seventeen to thirty-three days, with nodules at the point of inoculation, in the kidney, brain, and lung, and diffuse nodules in the peritoneum. From these nodules he got blastomycetes in pure cultures.

The nodules were composed of a delicate mesh of connective tissue surrounding and holding together a mass of blastomycetes, with very little surrounding inflammatory reaction. In the kidneys the blastomycetes were in heaps, chiefly in the glomeruli, surrounded by very little proliferated connective tissue. Only rarely did they extend into the kidney tubules, for the elastic tissue about the tubules was very resistant. At times the blastomycetes entered the protoplasm of renal epithelium and destroyed the cells, and sometimes they occluded large renal blood vessels and caused embolic necrosis of renal tissue.

In the liver blastomycetes never were seen, except very rarely in the blood vessels.

In the heart blastomycetes were sometimes seen in clots, or, very rarely, in the heart muscle.

In the brain often were nodules in the pia or in the brain substance. The surrounding tissue showed no reaction, but the masses of blastomycetes destroyed the adjacent brain elements. In the brain the capsule of the blastomycetes generally was wanting, and the blastomycetes were quite uniform in size.

In the lungs blastomycetes were single or in groups, generally in the interalveolar connective tissue. The lungs were largely atelectatic. In places alveolar epithelium was largely desquamated and included blastomycetes in their protoplasm. Proliferation of the connective tissue was almost entirely wanting.

Finally Busse,[5] in a monograph published in 1897, described his attempts to cultivate the bodies seen in cancer cells from human cancers and said that he failed. He succeeded in growing blastomycetes from nasal polypi, but believed that they were contaminations, and that they did not cause the growth. Moreover, injection of these blastomycetes into animals gave no result. He also got a blastomyces from a sarcoma. This organism was not pathogenic, except in one mouse, in which it produced a chronic inflammation of the lung. He got similar results from cancer of the lip and skin.

Subsequent to Busse's first article, but before the appearance of his *second* article which showed that the peculiar organisms he described came not from a "sarcoma," but from inflammatory reactive nodules, Sanfelice,[6] Professor of Hygiene at the Royal University at Cagliari, published a paper. Sanfelice was inclined to believe that the well-known cancer cell inclusions were blastomycetes from their morphology. Influenced by this idea, Sanfelice obtained a blastomyces from the juices of fruits which "came from the air," studied its cultural peculiarities, and inoculated animals. He found that guinea-pigs died in from twenty to thirty days with en-

larged lymph nodes, and with secondary nodules in the liver, kidney, and spleen, and was able to regain pure cultures of the organisms from the experimental nodules. Sanfelice believed that these blastomycetes were identical with the cancer inclusions. He found that the blastomycetes were most frequently in the lymph sinuses. Sanfelice called his organism "saccharomyces neoformans."

Sanfelice followed this publication with a series of articles on the same subject. In an article in November, 1895, Sanfelice[7] claimed that he had found in a primary cancer of the liver of an ox small refractile bodies, histologically similar to his saccharomyces neoformans, and some of these forms were surrounded by a deposit of phosphatic calcareous material. He was able to grow these organisms upon culture media containing sugar or starch, best on potato. This organism when inoculated into guinea-pigs caused death in two months, and produced nodules at the point of inoculation with enlarged lymph nodes. Sanfelice says that the nodules were due to an overgrowth of "fixed cells," without adjacent inflammatory reaction. The longer the inoculated animals lived, the less was the number of blastomycetes and the greater the amount of newly-formed connective tissue. In the lungs the alveolar epithelium proliferated as well as the connective tissue.

In a later article Sanfelice[8] summed up and generalized his previous observations. He stated that he had inoculated large numbers of a variety of animals with his blastomycetes, but in this paper are details of but two cases. Sanfelice claimed that the organisms he described in his previous paper were the same as the cancer cell inclusions seen and described by many men, and believed by them to be a protozoon and the cause of cancer.

Sanfelice used for inoculation a pure culture of his blastomycetes (neoformans) diluted in water. He inoculated a bitch in the breast, and the animal died in two months. At the point of inoculation came a hot, tender swelling which decreased in size one-half in four weeks, and remained stationary. The bitch was killed after three months. He found

at autopsy nodules in the kidneys and spleen, with large lymph nodes, but was unable to regain his organism in cultures. The primary nodule or site of inoculation was composed of "sarcoma" like tissue without adjacent inflammatory reaction, and blastomycetes were seen in the protoplasm of cells in this nodule. There was no proliferation of glandular epithelium.

Sanfelice also inoculated a cock in the comb. An immediate swelling resulted which later became indurated. The fowl was killed after three months, and it was seen that the nodule at the point of inoculation was composed of young connective tissue, while the blastomycetes were chiefly in the center of the nodule, and were usually in the protoplasm of the cells. There were many new blood vessels in the nodules.

Sanfelice then published an article in five parts on the pathological action of blastomycetes. In the first part[9] Sanfelice described the cultural peculiarities of his "neoformans," its morphology and the results of inoculation of guinea-pigs. He inoculated guinea-pigs in the testes, liver, abdomen, and connective tissue. He found that the animals generally died in thirty days with, at the point of inoculation, a nodule, like "fish flesh," which might ulcerate, with enlarged adjacent lymph nodes, and with secondary nodules in the kidney, liver, spleen and lungs, brain and cord, with blastomycetes in all of these nodules. The organisms showed a circular outline with double contoured membrane. In the center were refractile granules, with occasionally a hyaline zone outside the double membrane. Occasionally there were budding forms and hyphæ. In fresh scrapings from the nodules were many leucocytes containing blastomycetes. (Examination of the appended drawings show that these cells were mononuclear phagocytic cells and not polynuclear leucocytes.) In guinea-pigs the nodules consisted of young connective tissue in the meshes of which were lymphoid cells and blastomycetes without any inflammatory reaction. Some of the blastomycetes were free, while many were included in the protoplasm of cells, and there was no alteration of the adjacent skin and muscles.

Enlargement of the lymph nodes was due to the presence of parasites and not to an increase in the number of cells. The parasites in the nodes were chiefly in the lymph sinuses, but sometimes in the protoplasm of cells.

In the spleen the blastomycetes were chiefly in spaces, seldom in the follicles. In the bones there was an increase of lymphoid elements and blastomycetes often were in the protoplasm of leucocytes (!).

In the liver the blastomycetes were usually in the connective tissue about the blood vessels.

In the kidneys the blastomycetes were mostly in the cortex in a mesh of young connective tissue between the tubules, or in the vessels of the glomeruli.

In the lungs the blastomycetes were chiefly in the connective tissue of the alveolar walls, caused an increase of connective tissue, and finally compressed the alveoli.

In the brain, cerebellum, and cord they occurred rarely, but caused neither inflammation nor new growth.

In the second part [10] Sanfelice described the result of inoculating animals with his "saccharomyces litogenes." He inoculated ten guinea-pigs. The animals died in from one to two months with nodules in the spleen, omentum, rarely in the kidneys or liver, large lymph nodes, nodules in the lung, and no lesions in the nervous system. The litogenes produced in animals a calcareous deposit about the blastomycetes just as in the original animal (ox). The nodules were composed of young connective tissue, in the meshes of which were many blastomycetes, which lay chiefly in the lymphatic vessels.

Sanfelice also inoculated a sheep with the same organism and produced an abscess in which were many blastomycetes. The sheep recovered.

In the third part [11] Sanfelice described the results of inoculation of mice, white rats, rabbits, and dogs with his neoformans, and also claimed that he was able to obtain pure cultures of blastomycetes from human malignant tumors.

Mice inoculated with the neoformans died in eight days with a general saccharomycosis, from which in some cases

the original organism could be got in cultures. White rats did not react. Rabbits died in from thirty to forty-five days, and had nodules in the spleen, kidney, and omentum, but none in the liver. They showed more marked proliferation of connective tissue than did guinea-pigs.

Sanfelice also got pure cultures of blastomycetes from human tumors and from tumors of cattle and swine, and makes a most extraordinary statement regarding his technic which makes it seem probable that he also might have obtained pure cultures of blastomycetes from icicles. He says: "Wenn man auf solchen Platten zahlreiche Colonien von Blastomyceten findet, so kann man wohl sicher sein dass diese von den Geschwülsten und nichts etwa aus der luft herrühren, denn in den 5 oder 6 Stunden während welcher die Platten der Luft aufgesetzt waren, könnten sicher nicht so zahlreiche Blastomyceten auf die Platt gelangt sein." But Sanfelice obtained in this way no blastomyces which had pathogenic action upon animals.

In the fifth part [13] Sanfelice explained that the reason the organisms he obtained from human tumors gave no results when inoculated into animals is due to the fact that the conditions in animals are different, hence he inoculated a long series of animals with his neoformans, in order to "acclimate" the neoformans to animal life. Up to 1898 Sanfelice had inoculated fifty-nine dogs in a series and claimed that finally he obtained a "positive" result, *i.e.*, that he produced true cancer by the inoculation with blastomycetes. He inoculated the neoformans into the breast of a bitch. A swelling quickly resulted, which finally diminished in size, and then slowly grew again. The animal died in ten months and the skin over the nodule was adherent, inguinal lymph nodes were enlarged, but there were no metastases in the internal organs. The growth was an adenoma with an arrangement "like that in the normal breast." The lymph nodes showed some adenomatous metastases. There were no blastomycetes in the old part of the tumor, but there were a few in the periphery, some endocellular, but mostly free, but the *structure of these bodies did not correspond with the structure of the*

ordinary blastomycetes, because Sanfelice says, the longer the organism remains in the tissues the more it resembles the ordinary cancer inclusion (*sic !*) and in that stage it is incapable of culture upon ordinary media.

Sanfelice also inoculated a dog in both testicles with the neoformans after it had been passed through a series of animals. A tumor appeared on the penis which affected the glans, and there was a purulent discharge in which were bodies which looked like cancer bodies but were unlike ordinary blastomycetes. This animal died in six months. There were no metastases, and no blastomycetes could be obtained in cultures.

Sanfelice in the third part also described what he called a "successful case." In this case he inoculated a dog in the breast and killed the animal at the end of four months and found a "sarcomatous" nodule at the site of the inoculation. Sanfelice's own description of the histological appearance makes it certain that he had a nodule of proliferated connective tissue. (This was the same animal already mentioned in an earlier reference.)

Plimmer [14] claimed to have isolated from human cancer an organism which grew on special culture media anaërobically. He believed that this organism corresponded morphologically with the cancer cell inclusions. He inoculated animals with this organism. Some of these animals gave no results; in others the bodies could be seen in the tissues without reaction. Guinea-pigs inoculated in the peritoneum showed diffuse lesions in the peritoneum, omentum, and internal organs. Histologically these lesions were composed of "endothelial" tissues and Plimmer calls them "tumors." Plimmer said that the organisms "probably" were not protozoa but were allied to the saccharomycetes, but preferred to leave the matter of classification open at the time when his paper was written. Plimmer was able to regain his organism from the artificial nodules.

Following the articles of Busse and Sanfelice was a series of articles by various men, chiefly Italian observers. Many of these men accepted the theory that the inclusions seen in

human cancers were parasites, that they were the cause of the lesion, and that they were identical with the blastomycetes of Sanfelice.

Some of these men endeavored to prove by means of special methods and stains that the cancer inclusions were blastomycetes.

Roncali,[15] *e.g.*, gave a long description of the morphology of the inclusions seen in an adeno-carcinoma of the ovary, but was unable to isolate or cultivate his so-called "organisms."

Binaghi,[16] an assistant in Sanfelice's laboratory, examined fifty-three epidermoid cancers histologically and found blastomycetes in forty of them, although in some tumors they were in small numbers. He attributed his failure to find them in all to the fact that he was unable to examine the "entire tumor." He claimed that these bodies were blastomycetes because in form, color, and chemical reaction they were "identical" with blastomycetes, and he believed that they were the cause of the disease and not accidentally present because of their "regular distribution and their relation to the neoplastic cells."

Other men claimed to cultivate blastomycetes from human tumors.

Aievoli[17] found blastomycetes in human tumors, and cultivated them.

Mafucci and Sirleo[18] in an early paper examined a guinea-pig which died of marasmus after being inoculated with an embryo from a tubercular mother, and found blastomycetes in peculiar nodules in the lungs. These lung nodules showed, at the periphery, dilated alveoli in which were peculiar epithelial cells, then a zone of large alveoli filled with the alveolar cells and no leucocytes. In these epithelial cells blastomycetes were included. In the center of the nodule were alveoli filled with the blastomycetes and a few epithelial cells. The endocellular blastomycetes did not destroy the cells, but themselves underwent degeneration. The cells in which the blastomycetes were included showed no evidence of mitosis.

In the mediastinal lymph nodes were cells "like the epithelial cells in the lungs" in the lymph spaces, and Mafucci and Sirleo were inclined to believe that the alveolar epithelium had wandered into the lymph nodes. The blastomycetes were seen in the splenic follicles. In the kidney tubules were free blastomycetes which caused atrophy of the tubules.

Mafucci and Sirleo cultivated these blastomycetes and inoculated them into animals, and produced a nodule or ulcer in which were blastomycetes, free or enclosed, in endothelial or giant cells. Mafucci and Sirleo were not willing to say that these included blastomycetes were morphologically the same as cancer inclusions.

In a later paper Mafucci and Sirleo[19] gave the results of the inoculation of animals with the blastomyces they obtained from the tuberculous guinea-pig. They inoculated guinea-pigs, rabbits, fowls, and dogs.

In guinea-pigs they got nodules at the point of inoculation with secondary foci in lymph nodes, lungs, kidney, and spleen. The foci in the lymph nodes consisted of masses of blastomycetes and "epithelioid or epithelial" cells which replaced lymphoid cells. In the lungs the alveoli were compressed and filled with desquamated alveolar epithelium, some of which contained blastomycetes in their protoplasm. In some of the foci phagocytic giant cells were present. In the kidneys were nodules composed of epithelioid and giant cells which "seemed to arise from the tubular epithelium." In the older foci were blastomycetes with fewer epithelioid cells and the areas were infiltrated with leucocytes. In the spleen were foci of epithelioid cells, but blastomycetes were not present. The whole process was more like a new formation than like inflammation, but tended to heal. If the inoculation was in the abdominal cavity there were multiple metastases of a chronic inflammatory character.

In rabbits inoculations into the blood vessels often were negative, rarely they produced foci in the lungs. Inoculations into the connective tissue caused abscesses which ultimately healed. The lesions in the lung consisted of a

desquamative pneumonia, with the formation of giant cells and with an infiltration with leucocytes. In the kidney the lesions were chiefly in the capsule of the glomeruli.

Hens gave no reaction if inoculated in the connective tissue. If inoculated in the trachea there was a proliferation of tracheal epithelium and a formation of giant cells.

Dogs inoculated in the jugular veins had a general infection with metastases in lung, liver, kidney, pancreas, heart, and spleen, with enlarged lymph nodes. In the lung there was a desquamative pneumonia, with endothelial nodules in the pleura. In the liver were epithelial masses often infiltrated with leucocytes. In the kidney were nodules of epithelial cells, while the tubules were filled with formless epithelial cell masses. Often the center of the epithelial cell masses were necrotic. In the brain only the cortex was affected, and in the pia were masses of endothelial and giant cells with proliferation of the perivascular lymph spaces. In the spleen were masses of epithelioid cells.

Mafucci and Sirleo failed in their attempts to cultivate blastomycetes from human cancers, and expressed the belief that Sanfelice's results were accidental. But they believed on the whole that the nodules had the character of new growths, and often "were like cancers or endothelial growths."

They also found later[20] by inoculation of animals that the action of the blastomyces upon connective tissue and endothelium was transitory and led to necrosis of the tissue, and was then followed by acute inflammation and scar formation. They believed that the process was one of chronic inflammation and not of new growth. They examined ten non-ulcerated cancers to see if blastomycetes were present, but obtained negative results.

In another paper Mafucci and Sirleo[21] gave further results of their attempts to cultivate blastomycetes from human tumors. They examined thirty-nine tumors, cancers, or sarcomas, from men and animals, and got a growth of blastomycetes in six cases. With these blastomycetes they inoculated two hundred and twenty-six animals. Some varieties

of blastomycetes produced acute inflammations, others caused nodules of proliferated cells, but nothing approaching cancer. They found, however, that both the cultures and the filtrate of cultures were toxic, but that the filtrate caused no chemotaxis.

In the cancers in which they found blastomycetes they decided that living blastomycetes were in "inflammatory" cells, while dead ones at times were in the protoplasm of epithelial cells. They never obtained blastomycetes from fresh, living, aseptic, non-ulcerated tumors, but did find them in ulcerated tumors. Hence they believed that the blastomycetes represent an infection through an ulceration. Mafucci and Sirleo concluded that some blastomycetes are pathogenic, but cause only an inflammation, which may be suppurative or chronic; that blastomycetes can occur in human tumors, but that they mean a secondary infection, and are not the cause of the new growth. In spite of this they believed that some cancers are infectious, although it is not yet proven, while others represent a proliferation of embryonic inclusions. Mafucci and Sirleo believed that Sanfelice's "successful" cases could be explained as coincidences, especially as it is well known that dogs are susceptible to cancers.

Corselli and Frisco[22] made cultures from human tumors and obtained blastomycetes and inoculated animals with them and produced neoplastic nodules with enlarged lymph nodes. Their description of their histological findings is unconvincing and their drawings are even more so.

Leopold[23] claimed that he began to hunt for a parasite in cancer in 1894 in order to relieve the sufferings of his patients. He saw peculiar glistening bodies in cancers, and in these bodies were motile granules. Later he cultivated blastomycetes from four out of twenty cases of cancer of the pelvic organs. He believed that these blastomycetes were identical morphologically with the inclusions in cancer cells. Leopold also tried inoculation experiments. He inoculated a white rat in the testis with a pure culture of blastomycetes obtained from human cancer. At the point of inoculation there developed what Leopold calls a "very vascular giant

celled sarcoma," with multiple metastases of the peritoneum. In both the primary nodule and the metastases were blastomycetes. He also inoculated a white rat in the peritoneum with a bit of an ovarian cancer in which blastomycetes were present. A tumor developed in the groin of the animal like a medullary sarcoma, but no blastomycetes could be obtained on culture, although in the tissue were bodies like cancer bodies, with cells in groups, with blastomycetes between them. Hence Leopold calls this tumor an adeno-sarcoma (*sic !*). Leopold also put a bit of a uterine cancer into the belly of a rabbit and the animal died in four years, with a nodule the size of an orange in the abdomen, filled with pus, and also with nodules in the lung. These nodules in the lung showed "atypical epithelial growth."

Besides the above-mentioned men who, temporarily at least, accepted the theory that blastomycetes were identical with the cancer bodies, and were the cause of the epithelial cell proliferation, there were other men who found blastomycetes in human or animal inflammatory lesions.

Curtis[24] found blastomycetes in a soldier who had a nodule in the groin and a similar nodule in the back. The nodules clinically were supposed to be sarcomata, but at operation were found to be situated in the subcutaneous tissue without invading the deeper tissues. Curtis' organism was usually naked in cultures, but had a homogeneous capsule in the tissues. Curtis cultivated this blastomyces best on sugar, or on starch media, in the air, although it was not killed by an absence of oxygen. He inoculated guinea-pigs, rabbits, rats, mice, and dogs. The organism produced no lesion in guinea-pigs; in rabbits a nodule formed filled with pus, which finally disappeared. In white rats was produced a mass of new tissue. Such a new nodule might open, empty, and finally heal, or a general infection, with metastases in the lungs and kidneys, might result. In mice sometimes came multiple skin tumors, due to an infection with cocci. In dogs the result varied with the dose. Small doses produced a local nodule which at length disappeared; large doses produced a nodule filled with bloody fluid and blastomycetes.

The clinical appearance of the human nodules was peculiar. The mass was soft and had a mucous appearance on section, and at times the blastomycetes formed almost the entire bulk of the mass. The center of the nodule was composed of strands of connective tissue infiltrated with leucocytes and blood vessels. Outside of this was a zone of bundles of well-preserved fibrous tissue infiltrated with small cells. In the central zone the cells were about the blastomycetes and were epithelioid, and at times formed giant cells, which enclosed the blastomycetes. The included blastomycetes lost their gelatinous capsule. The nodules in rats were the same as in human beings, *i.e.*, "not a neoplasm, but a pure culture of the organism on the living being." In rats there was no zone of leucocytes, but the nodule formed without producing a marked reaction of the tissues. In the lungs the blastomycetes entered the alveolar walls, which thickened, and then the parasite might enter the alveoli, become surrounded by endothelial cells, and occlude the alveoli. Lesions in the rats' kidney were of similar tissue. Curtis' organism inverted sugar and caused fermentation.

Gilchrist's[25] case of blastomycetic dermatitis is well known. The case clinically was considered to be scrofuloderma. Histologically the lesion was chiefly in the rete of the skin, and consisted of miliary abscesses of the rete, in which were blastomycetes. There were also abscesses in the corium with proliferation of mesoblastic tissues, and an inflammatory exudate. At times giant cells were seen which might be phagocytic. Gilchrist says that no one could take the blastomycetes for psorosperms.

Since Gilchrist's paper, twenty-six cases of blastomycetic dermatitis have been reported, twelve of them by Ricketts[26] of Chicago. Ricketts, in a very elaborate monograph, gave an excellent resumé of the literature, and reported in detail complete clinical, cultural, and pathological observations. He stated that the lesions clinically have been taken for epidermoid cancer or skin tuberculosis. Enlargement of lymph nodes seldom occurred. Histologically the process consisted of a proliferation of the deeper layers of the epidermis, with

infiltration of all of the layers with leucocytes, and of an intra-epithelial infiltration. The epidermis generally formed deep processes, which projected downward into the corium. The corium also was proliferated, and contained minute abscesses. The organisms which produced the lesions varied in morphology, and could be blastomycetoid, oidium-like, or hyphomycetoid. From some of the cases, including one under observation for several months, it was impossible to obtain a growth of blastomycetes on culture media; in other cases cultures were obtained only from the pus, while there was no growth obtained from the tissues; in some a growth was obtained both from the pus and tissues. Animal experiments cannot be said to have been very successful. Many animals inoculated either with pure cultures or with bits of tissue showed no reaction. Sometimes an inflammatory nodule or a local abscess was produced with the organisms in the abscess; once there was a growth of the organism in the kidney of a mouse, and once the formation of metastatic nodules in the lung of a dog. Some of the experiments are not yet completed. Although the morphology of the various organisms isolated from these cases varied widely, Ricketts says that the morphology in the tissues of the different organisms was very similar. Ricketts also incidentally observed that although blastomycetes have been isolated from human cancers, there is no evidence that the changes produced by those organisms were anything else than inflammatory.

Besides the cases reported by Gilchrist and Ricketts, twelve other cases of blastomycetic dermatitis have been reported.

Fermi and Aruch [27] found blastomycetes in "lymphangitis epizootica," a disease of horses somewhat resembling glanders clinically. This disease was first described by Rivolta in 1873. The infection generally takes place through small wounds of the skin. The chief nodules generally occur in the skin, rarely in the mucous membrane of the nose. The nodule at first is small, hard, and painless, and may persist or may break down and ulcerate. Extension from this nodule generally is along the lymphatics, and leads to a swelling of

the adjacent lymph nodes ("rosary"). Sometimes the organism gets into the circulation, and metastases occur in the internal organs. In the air passages the lesions of the mucous membranes may extend to the nasal bones, or there may be ulcers of the air passages. About three-fourths of the animals in good condition which are affected recover, while animals in poor condition generally die. In the tissues blastomycetes always are present, and can be cultivated upon potato. Fermi and Aruch inoculated horses, rabbits, and guinea-pigs. In rabbits they produced local suppurations, while in the other animals they got no reaction.

Tokishige[28] found blastomycetes in a similar disease which occurs in horses in Japan. He states that the blastomycetes were free or in leucocytes (although his plates show that the including cells are not polynuclear leucocytes, but mononuclear phagocytic cells). In the soft nodules the yeasts may lie in phagocytic cells, and then grow at the expense of the cells, and then again become free. Tokishige inoculated a horse with pure cultures of his blastomycetes, and once obtained an inflammatory nodule which contained blastomycetes. Other inoculation experiments on the same horse, and on guinea-pigs, rats, and swine, were negative. Inoculation of animals by direct transplantation of tissue from diseased animals failed. Tokishige believes that the failure of these inoculation experiments is due to the fact that the bodies as seen in the tissues are perhaps only developmental stages of higher yeast forms, and that infection can occur only through the higher form.

Buschke[29] obtained yeasts from human skin ulcers, cultivated them and inoculated them again into the patient, and produced inflammations. Buschke also tried to obtain blastomycetes from human cancers and sarcomas. In one case a blastomyces did grow, but it did not occur in the original tumor, and was not pathogenic in animals. Hence Buschke decides that this blastomyces was due to accident, especially since lesions in men due to blastomycetes are exceedingly rare. Buschke examined sections from two of Roncali's cases, and declared that although the inclusions looked like

yeasts there was no proof that they were, nor was it even certain that the tumor was a cancer.

Buschke[30] had the patient mentioned in Busse's first article under observation for some months. There were ulcers on the face due to the blastomycetes. Buschke claimed that the skin lesions preceded the lesion in the tibia that Busse studied. Buschke considered the lesion one of chronic inflammation. He mentioned that the epidermis tended to grow downward into the corium, but he believed that this proliferation of epidermis was due, not to the action of the yeast, but to the chronic inflammation of the underlying subcutaneous tissue. In fact, when the blastomycetes came into contact with the epidermis they destroyed it. Buschke often found blastomycetes in the epithelial cell protoplasm. He believed that this was due either to ingrowth by the blastomycetes, or to their being carried in by leucocytes. Such epithelial cells were destroyed by the blastomycetes, and were not induced to proliferate. Buschke inoculated scratches in the skin of his patient with blastomycetes, but succeeded in producing a dermatitis only in the vicinity of the original lesion. Experiments on other parts of the body failed. Buschke believed that all truly pathogenic blastomycetes developed a gelatinous external capsule, but was unable to state the composition of the capsule, while ordinary "wild" blastomycetes cause only a septicemia and not a true blastomycosis. Buschke believed that as a rule the infection arose through minute lesions in the skin.

Colpe[31] saw a long continued endometritis which he claimed was caused by a blastomyces. Ernst[32] found blastomycetes in the urine of a diabetic patient, and in the pus in a perirenal abscess.

Finally a number of observers have inoculated animals with a variety of blastomycetes obtained in a variety of ways, and have carefully studied the histology of the experimental lesions. Some of the organisms thus studied were obtained from the air, some were pure cultures of brewery yeasts, and one set of observers had an opportunity to study the action of Sanfelice's "neoformans."

Neumayer[33] experimented with various yeasts and came to the conclusion that all yeasts injected subcutaneously are inert; that if eaten with vegetable food they are also inert, but if eaten without vegetable food they may cause a gastro-enteritis.

Nesczadimenko[34] inoculated many animals with yeasts of various sorts and found that they generally produced an abscess, the walls of which were composed of granulation tissue. He explains Neumayer's negative results by stating that none of the yeasts which Neumayer used could have been pathogenic.

Rabinowitsch,[35] in order to investigate the value of Sanfelice's claims, and, at that time, since Sanfelice had not completed his work, unable to obtain a culture of Sanfelice's "neoformans," carried out animal experiments with fifty different yeasts, obtained in various ways. Of these fifty yeasts only seven proved to be pathogenic in animals, ordinary yeasts being negative. Of the seven pathogenic yeasts three caused suppuration at the point of inoculation, and got into the general circulation. The other four produced abscesses at the point of inoculation. Rabinowitsch found that guinea-pigs did not react to the yeasts she used, but that the yeasts were pathogenic for mice and sometimes for rabbits. She produced an infection, but saw no evidence of intoxication. In the tissues the yeasts generally were between the cells, sometimes in them, and often there was a gelatinous capsule about the yeast. There was absolutely no true "tumor" formation.

Foulerton[36] used various blastomycetes and inoculated rabbits and guinea-pigs. He frequently produced nodules composed entirely of granulation tissue. He stated that the proof of the correctness of the theories of Sanfelice and Roncali was quite inadequate.

Petersen,[37] and Petersen and Exner,[38] gave the results of a series of experiments upon animals with a pure culture of Sanfelice's "neoformans." They found that the organism was pathogenic for mice and guinea-pigs, and produced large nodules at the point of inoculation, with metastases in

the kidney, spleen, and lung, almost no change in the liver, while the lymph nodes were enlarged. In the kidney the blastomycetes destroyed the glomeruli, while the canaliculi were filled with blastomycetes, and at times the urine was milky and contained blastomycetes. The renal cells surrounding these areas were destroyed by pressure, but there was almost no trace of acute inflammatory reaction. In the skin the nodules were composed of highly developed granulation tissue in which were many giant cells and masses of blastomycetes. Nowhere was there anything approaching the structure of sarcoma or carcinoma. These men concluded that although some blastomycetes are pathogenic for animals and men they are not analogous with cancer bodies, and that the experimental lesions produced by injection of blastomycetes are nodules of granulation tissue.

In comparison with the results of many men who claimed to find blastomycetes in human tumors with facility and frequency, the results of Richardson's [39] experiments are very striking. He examined forty tumors bacteriologically, using all the ordinary culture media and many media especially prepared. From no tumor did he succeed in growing any variety of blastomycetes.

Bonome [40] examined twenty-six cancers for blastomycetes, but found yeasts in but six cases. He concluded that blastomycetes occurred but seldom in such tumors, and more often in those which were ulcerated and exposed. He believed that there was no proof that they were the cause of the tumor growth, but that they were probably an accident. Some of the blastomycetes so obtained were pathogenic for animals, but produced only a new formation of connective tissue. He also stated that pure cultures of pathogenic yeasts injected into living cancers produced softening and degeneration of the tumors. He believed that blastomycetes did not produce cancers.

Sternberg,[41] working from the histological side, examined the inclusions in cancer cells to see if their morphology corresponded with the morphology of true blastomycetes. He got wretched results with Roncali's and Sanfelice's technical

methods (as did the writer of this article). Sternberg saw various types of inclusions which may fairly be said to correspond to those inclusions in cancer cells described by various adherents of the blastomycetic theory, and claims that the inclusions are vacuoles in which there are leucocytes, or areas of mucous degeneration with contraction, thus giving rise to a rayed appearance, or areas of calcification, or included red blood corpuscles. He also showed that Sanfelice's so-called "specific" stain for blastomycetes is but an unessential modification of Gram's stain, and gave a long list of the different tissues and degenerations which Gram's stain will color. He also stated that he and several men had found bodies analogous to cancer bodies in other lesions than cancer and sarcoma. Also real blastomycetes did not stain as do the cancer bodies. Hence Sternberg concludes that the presence of the cancer bodies in cancer cells has led us to study the minute histology of cancer cells, but that as yet we know nothing of the etiology of cancer.

Moreover, even if the cancer cell inclusions were really blastomycetes, it has been shown that they are not constantly present in cancers; *i.e.*, they are not present in such numbers or in such relation to the pathological process as fairly to be considered as a causative agent. Greenough[42] found cancer bodies in twenty-three cases of cancer of the breast. He failed to find them in one case of Paget's disease, in a peritoneal cancer, and in three epidermoid cancers. They were more numerous in slow-growing cancers than in those which grew rapidly.

Nichols[43] examined twenty-one cases of alveolar cancers of various organs, and in five cases was unable to find cancer bodies. In fourteen epidermoid cancers cancer bodies were seen in but one case. In five sarcomas cancer bodies never were present.

It also must be remembered that the mere occurrence of blastomycetes in human malignant tumors is no evidence that the blastomycetes are the cause of the cancer. De Meser[44] examined an epidermoid cancer of the arm and found in the stroma peculiar three-cornered bodies with a dense

skin, containing granules. He finally determined that these bodies were lycopodium spores, and inquiry showed that the patient had been in the habit of dusting the malignant ulcer with lycopodium. Sometimes these bodies were surrounded by masses of epithelial cells, and the spores at times lay in the middle of the tumor, showing either that they had been taken in by granulation tissue, which is unlikely, as the spores were too large to travel in the lymphatic vessels, or that the epithelial cells of the cancer had grown up above the adherent lycopodium powder. De Meser says that if lycopodium spores can be taken into a cancer it would be an easy matter for blastomycetes to be taken in the same way, and that even if blastomycetes do occur in human cancers they are not constantly present, nor are they sufficiently numerous, or so related to the lesion as to be a probable cause of the disease.

Experiments with Sanfelice's Neoformans and Plimmer's Organism.

My investigations have been confined to a study of the lesions produced by the inoculation of animals with Sanfelice's "neoformans" and with the organism ("saccharomyces") isolated by Plimmer from a human cancer. Two years ago I had an opportunity to examine the work of both of these gentlemen in their own laboratories, and each furnished me with a pure culture of his organism. I wish here to thank both Professor Sanfelice and Mr. Plimmer for their kindness.

Each organism was transferred to various media, and grown in pure cultures. Both organisms grew rapidly aerobically on various sugar media, on potato, and in glucose bouillon.

Thirty animals in all were inoculated, eighteen with Sanfelice's "neoformans," twelve with Plimmer's organism. Of those animals inoculated with the "neoformans" six were rabbits, the others guinea-pigs. One animal inoculated with Plimmer's organism was a rabbit, the rest were guinea-pigs.

For inoculation a culture in glucose bouillon was invariably used. The culture tube was agitated until the growth was

disseminated throughout the bouillon, and then a small amount, generally from .25–1 cc., of the culture was injected into the tissues. There was no appreciable variation of the action produced by cultures of the same organism of different ages, although some of the cultures were two or three months old.

The inoculations were made in various places, including the subcutaneous tissue, the anterior chamber of the eye, the testis, breast, nipple, peritoneal cavity, and blood vessels.

Gross Lesions. Subcutaneous Tissue. — Four animals were inoculated subcutaneously with the "neoformans." In every case there was well-marked local reaction, appearing in from four to seven days. In one case the reactive nodule attained a diameter of 1.5 cm., then diminished in size, and ultimately disappeared. Two nodules were tense and elastic, but did not fluctuate. One nodule after growing rapidly and attaining a large size showed obvious fluctuation, and on section showed a distinct abscess cavity, filled with pus, blastomycetes, and phagocytic mononuclear and giant cells containing blastomycetes. The nodules on section were at times myxomatous or even gelatinous, or else dense, firm, and rather pale.

In every case the adjacent inguinal lymph nodes were enlarged. In some nodes the consistency was increased, and on section they were firm and pale. In other cases the nodes were very large, soft, and edematous, myxomatous or gelatinous on section. In no case was there any definite abscess of the nodes, but occasionally small yellow areas of softening were seen.

Five animals were inoculated subcutaneously with Plimmer's organism. In each case a reactive nodule appeared at the point of inoculation. In one case this nodule attained the size of a large bean and ultimately disappeared, although the adjacent lymph nodes enlarged and remained so. In the other cases a nodule developed at the point of inoculation which increased during the life of the animal at a more or less rapid rate. In two cases this nodule was adherent to the abdominal muscles, and pushing the peritoneum ahead

of it, formed a flattened papillary mass which projected into the abdominal cavity. The consistency of these nodules varied from firm and dense to very soft and myxomatous. In all of these cases the lymph nodes in the inguinal region were enlarged.

Testicle. — Four animals were inoculated with the " neoformans " in the testicle. In each case there was a reaction which resulted in an enlargement of the testicle. In each case the nodule enlarged steadily during the life of the animal, and might attain a size two or three times that of a normal testis. In one case the nodule was softened in the middle, and showed a cavity filled with bloody pus. In the other cases the nodule was solid, showed no caseation, and was edematous or myxomatous in appearance on section. The adjacent inguinal lymph nodes were enlarged.

Three animals were inoculated in the testicle with Plimmer's organism. In each case a reactive nodule appeared. In one case this nodule attained the size of a bean and then slowly receded, but never entirely disappeared. The other nodules constantly increased in size while the animal lived. In one case the nodule involved the testis and the skin of the scrotum and formed an enormous nodule five centimeters in diameter. In none of these cases was there any abscess formation. On section the nodules were pale, edematous, myxomatous, or gelatinous. Inguinal lymph nodes were enlarged, and their gross appearance was the same as that of the nodes in the cases of the subcutaneous injection.

Breast. — Four female animals, three guinea-pigs and one rabbit, were inoculated in the breast with the " neoformans." In two cases the inoculation was made into the ducts of the nipple with a fine needle. In the other cases the injection was made directly into the breast tissue. In every case a reactive nodule appeared at the site of inoculation. In one case the nodule attained its maximum size, then slowly receded. In the other cases the nodule increased in size while the animal lived. In the two cases in which the injection was made into the nipple, the swelling came first in the nipple itself, later the nodule extended to and involved the breast.

In one case the nipple became black and dry, and finally sloughed off, but the ulcer healed. In every case there was a swelling of the adjacent lymph nodes. The character of the breast nodule on section corresponded to and varied as did that of the subcutaneous nodules. In no case was there abscess of the breast nodule, but in one case there was necrosis and softening of the centre of two inguinal lymph nodes.

Two animals were inoculated in the breast with Plimmer's organism, and a reactive nodule with large lymph nodes formed, similar to those produced by the "neoformans."

Peritoneal Cavity. — Three animals were inoculated in the abdominal cavity with the "neoformans." In one case the injection was made through the abdominal wall and .25 cc. of a bouillon culture was injected through a subcutaneous syringe. In the other two cases an incision was made through the abdominal wall and a drop or two of a similar culture was dropped into the abdominal cavity just below the lower surface of the liver. In the former case there was a fibrinous peritonitis, with blastomycetes in the pus. In both of the latter cases there was a marked local inflammation with adhesion of intestinal folds to the liver and abdominal wound.

Two animals were inoculated in the abdominal cavity with Plimmer's organism — one through the abdominal wall with a hollow needle, and one through an abdominal incision. One animal showed local inflammation with adhesion of intestinal folds to the abdominal scar. The other animal showed, disseminated throughout the abdominal cavity, small circular nodules, the size of the head of a pin, translucent and vesicular. In these nodules blastomycetes could be found in the fresh state. There also was an adhesive peritonitis.

In all of the animals inoculated in the peritoneum there were metastases in the internal organs, with enlargement of inguinal and retro-peritoneal lymph nodes.

Anterior Chamber of the Eye. — Two animals were inoculated in the anterior chamber of the eye with one drop of a culture of the "neoformans." In both cases a hypopyon

developed, ultimately the cornea ruptured and discharged pus in which blastomycetes were present, and the eye-ball became filled with a mass of edematous granulation tissue. In one of these cases the eye was the only place inoculated in the animal, and no metastases occurred. In the other case a subcutaneous inoculation was made in the same animal and metastases in internal organs did occur.

Blood Vessels. — Two rabbits were inoculated in the ear veins with the "neoformans." In both cases there was a local nodule of reaction at the point of inoculation, and in one case this nodule broke down, opened, and discharged pus in which blastomycetes were present. Ultimately the sinus healed. One animal was killed at the end of seven weeks. There was one small, circular translucent nodule in the kidney; the other internal organs were normal. The second animal lived four months, and at autopsy showed small areas of proliferated mesoblastic tissue in the lungs in which were a very few degenerated yeasts, and also showed a few small scars in the kidneys in which no yeasts were found. The other internal organs showed no lesions.

Metastases. — Besides the local reaction produced at the point of inoculation, all of the animals above mentioned except six gave evidence of dissemination of the blastomycetes in the internal organs. Of the six, one was the guinea-pig that was inoculated in the anterior chamber of the eye. The eye ulcerated and was filled with a mass of edematous granulation tissue. There were no internal metastases. One guinea-pig inoculated with the neoformans in the abdominal cavity through an abdominal incision showed a localized abscess with adhesions in the abdominal cavity, a peculiar ulcerating scar, and no internal metastases. One guinea-pig, inoculated in the breast with the neoformans, lived two and one-half months and showed no infection of the internal organs. One guinea-pig inoculated in the breast with Plimmer's organism had a local reactive nodule form which finally nearly disappeared and the internal organs showed nothing. One rabbit inoculated in the testicle with Plimmer's organism had a reactive nodule in the testis which

finally nearly disappeared, and there was nothing in the internal organs.

All of the other animals showed more or less evident areas of proliferation in the internal organs, and in these areas blastomycetes were present. In one case, a rabbit inoculated in the ear-vein with the neoformans had a local abscess at the point of inoculation. The animal was killed at the end of seven weeks, and the only evidence of metastasis was one small nodule in the kidney. In all of the other animals the metastases were well marked. In few cases the metastases were found only in lung or kidney, but generally the metastatic lesions were found in lungs, kidney, and spleen. In three cases there were areas of proliferation in the pia of brain and cord, with metastases in brain and cord. The metastases were always confined to the perivascular lymph sheaths of the blood vessels. In only two cases were areas of proliferation seen in the liver substance.

Gross Appearance of Organs. Liver. — In two cases the liver showed changes in its substance. In one case the local lesions appeared as small, rather translucent, nearly circular nodules in the liver tissue. In one case the liver showed very extensive gross changes. The liver was much injected, and scattered through it were numerous irregular areas, variable in size, circular or irregular in outline, with crenated edges, yellowish or white in color, firm and dense to the touch, with occasionally small yellow areas of softening in the centre. In two other cases in which there was direct extension to the liver from adjacent organs small areas, about the size of a pin's head, circular and rather translucent, were seen on the surface of the liver.

Heart. — In but one case did the heart show a metastasis. In that case a small, circular, clear vesicular nodule, about the size of a pin's head, was seen beneath the serous covering of the ventricle. From that vesicle blastomycetes could be obtained in the fresh state.

Lungs. — The lungs frequently were atalectatic even when no metastases or blastomycetes could be found on histological examination. At times considerable areas of the lung

were injected, and denser than normal and slightly depressed below the level of the rest of the lung. Sometimes on the surface of the lung were depressed pits, irregular in outline, pale and rather firm on section. Frequently on the surface of the lung, beneath the pleura, were small, clear, circular vesicular nodules, the size of the head of a pin. From these nodules blastomycetes could be obtained.

Kidneys. — Frequently, even when metastases were present histologically, the kidneys showed no gross change. Often, however, on the surface, beneath the capsule were small, circular, transparent vesicular nodules, the size of a pin's head, from which blastomycetes could be obtained.

Spleen. — Sometimes, even when metastases were present histologically, the spleen showed no gross change, but as a rule the spleen was large, pale, and rather dense, and studded with firm granular nodules of the size of a pin's head, due to enlargement of the Malpighian follicles. At times the nodules were similar to those seen on the surface of the kidney. Occasionally the spleen was studded with circular nodules, opaque and yellow, sometimes coalesced so as to give a crenated border to the nodules, and closely resembling tubercular lesions.

Abdominal Organs. — Sometimes small nodules, similar to those seen on the surface of the kidney, were seen in the omentum, in the mesentery, on the peritoneum, and on the surface of the intestine.

Clinical Symptoms. — Swelling at the point of inoculation appeared in from four to seven days. Swelling of adjacent lymph nodes was obvious in from ten days to two weeks. As a rule the animals gradually became emaciated, although a few animals with well-marked local reaction and, as the autopsy showed, little or no internal dissemination of the organisms displayed no obvious change in their physical condition. Three of the animals showed more or less extensive motor paralysis, and in these cases there was a proliferation of the pia with cerebral metastases. In one case the urine was very cloudy and almost purulent in appearance, and many blastomycetes were found free in the urine. The

duration of life of the animals varied. Some guinea-pigs soon became ill, and died in from ten to twenty-five or thirty days. Some animals, however, were very resistant. One guinea-pig, inoculated with the neoformans, had a well-marked local nodule which, however, ultimately disappeared, and the animal was killed at the end of ten weeks. In this case there were no metastases. Rabbits as a rule were more resistant than guinea-pigs and lived from four to six weeks. One rabbit, inoculated with Plimmer's organism, had a local nodule which disappeared, and the animal was killed at the end of five months. One rabbit inoculated with the neoformans died at the end of eighteen days with a general infection with blastomycetes.

Cultures from Animal Tissues. — Cultures were made from all animals in which there was well-marked reaction or metastases. Cultures were made upon glucose bouillon or on potato. Almost without exception it was possible to obtain pure cultures of the injected organism from the infected tissues. In but two cases, however, was it possible to obtain the organism in cultures made from the heart's blood.

Technic. — Tissues for histological examination were hardened invariably in Zenker's fluid. Alcohols of different strengths, formalin, and corrosive sublimate were tried at first, but the results with Zenker's fluid were so superior that the other fluids were given up. The sections were cut in paraffin. The best stain was the ordinary methylene blue and eosin. For some purposes the following stain was found valuable:

1. Ten per cent aqueous solution ferric chloride, two minutes. Drain and blot on slide.
2. Aqueous solution hematoxylin (one to two per cent), freshly made, two minutes.
3. Wash in water.
4. One per cent solution ferric chloride until blue color is removed from protoplasm and nuclear stain is distinct (watch under microscope).
5. Wash in water.
6. Anilin oil gentian violet, five minutes.

7. Wash in water.

8. I K I solution, two minutes.

9. Blot perfectly dry.

10. Alcohol ninety-five per cent to decolorize (watch under microscope).

11. Wash in water.

12. Aqueous solution acid fuchsin, one per cent, one part; saturated aqueous solution picric acid, two parts. Two to five minutes.

13. Wash in water.

14. Alcohol ninety-five per cent, three changes, blotting dry between each change.

15. Xylol.

16. Mount in Xylol balsam.

This method gives very beautiful results. Blastomycetes are stained a bright purple, nuclei are black, the protoplasm of the cells is a pinkish yellow, and connective tissue is red.

Besides these two methods, various stains for, *e.g.*, fibrin and connective tissue were used. On the whole the most useful stain for histological details was methylene blue and eosin.

Histology. — So far as the histological appearances were concerned, the changes produced in the tissues by the neoformans and by Plimmer's organism are so exactly similar that in the descriptions no attempt will be made to distinguish between them. The morphology of the two organisms in the tissues is so similar that it is impossible from histological examination to tell with certainty with which organism one is dealing; although with Plimmer's organism the average size of the blastomycetes seems to be a trifle larger, very large forms are perhaps somewhat more common, and a tendency to form chains of six to eight individuals is much more marked.

The general result produced by the blastomycetes in the tissues is the formation of nodules composed of new-formed tissue, which forms a mesh of tissue in the interstices of which varying numbers of blastomycetes are included. Often in this new-formed tissue there is almost no infiltration with

lymphoid or plasma cells, or with polymorphonuclear leucocytes; at other times infiltration with some or all of these cells is marked, and sometimes large areas of necrosis and solution and leucocytic infiltration, *i.e.*, true abscess cavities, are present. At times the proliferation of connective tissue is marked, and the number of blastomycetes present in the tissues is actually or relatively small. In many cases, however, the amount of newly formed tissue is very small, and the blastomycetes are present in enormous numbers, so that the greater portion of the nodule is made up of the organisms.

In all organs the cells which compose the newly formed tissue are apparently of two kinds, *i.e.*, strands of more or less dense fibrous or connective tissue, which form as it were supporting trabeculæ of the nodule. Between these trabeculæ are masses of round, oval, or more often polygonal cells, which have a vesicular nucleus, a finely vesicular lightly staining protoplasm, and a perfectly definite cell membrane, and these cells closely resemble endothelial cells. Some of these cells have more than one, often many nuclei (giant cells), and large numbers of these endothelioid cells are phagocytic, and include one or several more or less necrotic and faintly staining blastomycetes in their protoplasm. In no case, except occasionally in the lungs, is there any evidence of epithelial cell proliferation. Newly formed blood vessels often are seen, but they seldom are very numerous.

Morphology of the Parasites in the Tissues. — The blastomycetes vary greatly in size and staining reaction. The outline is circular nearly always. In the center is a clear space, refractile and homogenous, in which often are granules, and this space may be nearly colorless or may take a light stain, sometimes with the nuclear, sometimes with the protoplasmic stain. The periphery is marked by a definite membrane, usually showing a double contour. As a rule the outer ring is wider and takes a deeper stain than the inner. Between these two rings is a clear refractile zone, which, however, generally is darker than the central clear space. The gran-

ules in the clear central space may take either a nuclear or protoplasmic stain. At times these granules have a peripheral arrangement, just inside the inner ring; again they are scattered irregularly throughout the central clear space, or they may be collected in a ball in the center, or eccentrically. The large forms frequently take the nuclear or Gram stain so intensely that they appear only as large homogeneous circular bodies, in which no definite outlines can be distinguished. The very small forms appear as circular bodies with a central dot, surrounded by a circular space and a single-contoured cell membrane. These small forms often take only the protoplasmic stain. As a rule, in the tissues, outside of the double-contoured cell membrane is a clear, approximately circular space, so that the blastomyces appears to be lying in a vacuole between the mesoblastic cells. In some cases this secondary zone is not like a vacuole, but is filled with a translucent gelatinous homogeneous material which takes a faint stain, generally with the nuclear, rarely with the protoplasmic stain, *i.e.*, outside the cell membrane proper in the tissues is a clear zone or capsule of some gelatinous material, which as a rule is not seen outside of the blastomycetes in cultures. Sometimes, however, in old cultures many organisms can be seen which possess a similar capsule. The very constant appearance of this capsule about the blastomycetes in the tissues suggests that this capsule may represent a secretion due to blastomycetic action. At times in the vacuole-like clear space are fine rays, spines, or processes extending from the membrane of the blastomyces radially toward the periphery of the space. These radial processes probably represent contraction of the gelatinous capsule by hardening reagents. The blastomycetes more often are free in vacuoles between the newly-formed mesoblastic cells. Many, however, are included in the protoplasm of phagocytic cells. Most of the phagocytic cells are of the endothelioid type. Phagocytosis of epithelioid cells which are certainly derived from proliferated connective tissue, *i.e.*, which have no definite cell membrane and have a definite intercellular fibrillar substance, is not very uncommon.

The blastomycetes which lie in the phagocytic cells frequently do not show any remnant of the peculiar gelatinous capsule, although blastomycetes in which the capsule persists often are seen. As a rule the included blastomycetes are apparently necrotic, their outline is not so clear as when they are free, and they generally, even when of large size, take chiefly a protoplasmic stain. One or several (eight to ten) blastomycetes may lie in the protoplasm of a single phagocytic cell, and such included blastomycetes may be of various sizes and ages. In no case, however, is thère any evidence of budding by included blastomycetes, nor is there any evidence that the presence of blastomycetes in an endothelioid cell causes mitosis of the cell. At times the phagocytic endothelioid cells are multinuclear (giant cells), and often the nuclei of such a cell have a marked mural arrangement. Almost always the nucleus of the phagocytic cell takes a sharp nuclear stain, but at times it is slightly necrotic.

Comparison of Yeast Morphology with Morphology of Cancer Bodies.

It is obvious that, in dealing with a spherical organism of microscopic size occurring in the cells of hardened tissues, forms may be met with occasionally which it may not be possible to distinguish with certainty from circular parasites of an entirely different nature. But in examining these blastomycetes in the tissues one thing is very striking and that is the very definite morphology of the parasite and the great facility with which it can be recognized.

The same statement does not apply to the so-called "cancer bodies." In the protoplasm of cancer cells are small circular bodies which are described as having a central dot, a clear central space, and a double-contoured membrane. In fact, the pleomorphism of these inclusions is very great, as can be seen by examining drawings by various men who have based their conclusions as to the nature of the cancer bodies upon their morphology and staining reactions. It is obvious that although all of these men have seen similar

bodies in cancer cells, different men also include very different structures in their descriptions of cancer bodies.

In fact, the " cancer bodies " do appear as circular bodies with a central dot, a clear space, and a dark periphery. The central dot may be very small, take the same stain as, only in a less degree than, the central space, or part of it may take a nuclear stain, or it may consist of a mass of spherical granules, or may be a large circular homogeneous or granular mass which occupies the greater portion of the clear space. Or the central dot may be absent. The clear space may be homogeneous, or may show radiate processes extending from the centre to the peripheral membrane, as if the contents of the space had been contracted by hardening reagents. The periphery may with low powers appear double contoured, but with very thin sections, a perfect stain, and very high powers the true "double contour" so distinctly seen in yeasts practically never is seen. The periphery often shows a pecular reticular radiate appearance. I never have seen anything about cancer bodies which suggests the gelatinous capsule so constant in the neoformans and Plimmer's organism.

The distinction between cancer bodies and true blastomycetes is difficult to express in words, but to one who has worked with both the difference is very marked and it seems impossible under ordinary conditions that the one could be mistaken for the other. From a personal examination of Professor Sanfelice's specimens of cancer bodies and blastomycetic infection I am convinced that his belief that the morphology of cancer bodies and blastomycetes is the same is due largely to extremely poor technic.

Primary Nodule.— In connective tissue the reactive nodule generally consists of myriads of blastomycetes held in a mesh of newly formed connective tissue and endothelioid cells with a few young blood-vessels. The framework of this mesh is made up of bands or trabeculæ of fairly dense fibrous tissue composed of spindle-shaped cells with much intercellular fibrillar material. These trabeculæ enclose irregular areas, varying in size, in which lie masses of blastomycetes, nearly

all of which show a gelatinous capsule. Between these masses of blastomycetes are many large cells, oval, polygonal, or very irregular in outline, which have a vesicular nucleus and a finely vesicular protoplasm. At times these cells are cut flatwise so that the contour and structure of the cell can be made out; at other times the cells are cut edgewise, and show only thin processes between adjacent blastomycetes. At times nearly every one of these endothelioid cells is phagocytic, and encloses 1–n blastomycetes of varying ages. The included blastomycetes usually do not stain as sharply as the free ones. Some of the included blastomycetes may, while others do not show any trace of the gelatinous capsule. Among and between the blastomycetes and endothelioid cells often are seen polymorphonuclear leucocytes. Occasionally a phagocytic cell contains a leucocyte or a leucocyte and a yeast. In such nodules young blood-vessels usually are relatively few. The relative amounts of fibrous trabeculæ and endothelioid cells vary greatly in different nodules; at times young connective tissue preponderates, at other times the mass almost entirely is composed of endothelioid cells and blastomycetes. At times in the nodules are seen large areas of necrosis of the new tissue and in such cases the infiltration with polymorphonuclear leucocytes is great. Often in the fibrous trabeculæ is a well-marked, but not extreme infiltration with lymphoid and plasma cells. If such a subcutaneous nodule adjoins voluntary muscle fibres bundles of striated muscles may be seen surrounded by young myxomatous connective tissue, and the muscle fibres are necrotic and vacuolated. There is no invasion of the muscle fibers themselves by the blastomycetes. Budding blastomycetes are not seen in phagocytic cells. Sometimes eosinophilic leucocytes are numerous in the nodules. At times short chains of blastomycetes are seen, consisting of from six to ten individuals. The softened areas in the newly formed tissues may show necrosis and an acute inflammatory exudate, or may show actual abscess cavities. One rabbit, inoculated in an ear vein, had an abscess form at the point of inoculation. The abscess opened and discharged and the

wound healed. Histological examination (Plate XXV., Figs. 8 and 9) of this area showed small areas of necrosis and softening, with marked infiltration with polymorphonuclear leucocytes, surrounded by a zone of rather dense cells, mostly polygonal in outline, having a vesicular nucleus with an occasional delicate fiber of connective tissue between the adjacent cells. Very rarely in one of these polygonal cells could be seen the faint circular outline of a necrotic yeast. Surrounding this zone of polygonal cells was a zone of very dense fibrous tissue. Besides the large areas just described there were occasionally small masses of the polygonal cells between the fibrous tissue bundles. The picture suggests that the polygonal cells may have arisen from proliferation of endothelial cells lining lymph clefts in the fibrous tissue.

Primary Nodules in Epithelial Organs. Testicle.

In the testicle the proliferation of tissue is confined entirely to the intertubular connective tissue. In many cases the glands of the testicle show practically no alteration, but the connective tissue between them shows enormous proliferation with the formation of myxomatous young connective tissue, a few young blood vessels, and many endothelioid cells. In the meshes of this tissue are many blastomycetes, some free with the usual gelatinous capsule, others enclosed in the protoplasm of phagocytic cells. Usually the membrana propria of the tubules appears to be able to resist the invasion of the blastomycetes. There is no evidence of increased proliferation in the tubules, nor of phagocytosis by the tubular epithelium, although occasionally a tubule is seen in which a few blastomycetes are lying free. Sometimes all of the tubular epithelium becomes necrotic, and the tubule is represented only by a tubule, with parallel walls of unruptured basement membrane, filled with granular and fibrillar detritus. In the proliferated areas lymphoid and plasma cells usually are fairly numerous, with occasionally eosinophiles. Occasionally the blastomycetes penetrate the basement membrane, push between the epithelial cells and raise them up from the basement membrane, and so get into the

tubules. In such tubules thus penetrated leucocytes generally are numerous. Occasionally in nodules in the testis and epididymis are true abscesses in the proliferated areas, and such abscesses may be surrounded by a wall of granulation tissue consisting of fibroblasts, endothelioid cells, and young blood vessels, outside of which may be a zone of very dense fibrous tissue.

Breast. — The changes produced by the blastomycetes are, as in the testis, almost entirely confined to the intertubular connective tissue. This tissue shows great proliferation with the formation of fibroblasts and endothelioid cells, and a few young blood vessels. In the meshes of this tissue many blastomycetes are included, some free and many in phagocytic cells. In these areas are fairly numerous lymphoid and plasma cells, with an occasional eosinophile. The phagocytic cells are often polynuclear. Occasionally blastomycetes are seen free in tubules and ducts, but there is no evidence of increased proliferation or phagocytosis of the glandular epithelium. At times glands are dilated and filled with a hyaline or mucoid material. Sometimes the proliferated intertubular connective tissue projects into dilated glands, pressing the epithelium ahead of it, and produces an intra-canalicular appearance. The glands usually are widely separated by the newly formed intertubular tissue and by masses of blastomycetes. Occasionally the blastomycetes, as in the epididymis and testis, penetrate the basement membrane of the glands, push between glandular epithelium or raise it from the basement membrane, and so enter the ducts. In one guinea-pig which lived ten weeks the blastomycetes had nearly disappeared. The breast nodule was small. The periphery (Plate XXXI., Fig. 1) of the nodules showed the glands separated by a moderate amount of edematous connective tissue in which eosinophiles were fairly numerous, with a few lymphoid and plasma cells, among which no blastomycetes could be seen. Nearer the center of the nodule the glands were more widely separated by masses of fibroblasts and endothelioid cells, with fairly numerous lymphoid and plasma cells and young blood vessels. Among the cells

were very numerous giant cells. In this tissue were a few blastomycetes chiefly intracellular, and usually in giant cells. Most of these blastomycetes were necrotic. Some of the blood vessels showed marked endarteritis.

Lymph Nodes. — The histological appearance of the lymph node depends upon the amount of infection with blastomycetes and the duration of the process; but the general process always is the same, *i.e.*, an invasion of the lymph sinuses, a desquamation and proliferation of the endothelium lining the sinuses, and ultimately a proliferation of the stroma of the node and a destruction of the lymphoid tissue. In the earliest stages the changes appear in the lymph sinuses, generally in the peripheral sinuses. In the very earliest stages the lymph sinuses are dilated, and contain many leucocytes and much fibrin, with blastomycetes in the sinuses. Some of these blastomycetes may be free, others may be included in the protoplasm of the endothelial cells of the sinuses. Desquamated endothelial cells also are common. At this stage an occasional free blastomyces may be seen among the lymphoid cells of the node. In the later stages there appears a new formation of tissue, at first most marked along the course of the lymph sinuses. The proliferation affects the stroma of the lymphoid areas, and results in the formation of a combination of spindle-shaped cells with intercellular fibrillar substance, and many endothelioid cells such as are seen in the primary nodules. The connective tissue cells appear to rise from the trabeculæ and reticulum, the endothelioid cells from proliferation of endothelial cells. Many of the endothelioid cells are phagocytic. Many blastomycetes may be seen free in clefts between the newly formed cells. In this stage an acute inflammatory exudate usually is wanting. At times the sinuses are everywhere dilated and filled with enormous numbers of free blastomycetes, about each of which is a gelatinous capsule. Often large islands of the lymphoid tissue are seen surrounded by the newly formed cells, and in such areas the cells are almost entirely plasma cells, with practically no lymphoid cells remaining. As the areas of proliferation enlarge it is not uncommon to

find foci of necrosis and infiltration with polynuclear leucocytes. In the proliferated areas may be many eosinophilic cells. At times the sinuses are practically occluded by proliferated endothelial cells, many of which are phagocytic and enclose blastomycetes. Occasionally the endothelioid cells in the sinuses contain several nuclei (giant cells). Occasionally the endothelial cells lining the sinuses show two nuclei, and mitotic figures are not uncommon.

As the process continues the node becomes larger from the presence of newly formed tissue and myriads of blastomycetes. The lymphoid cells may absolutely disappear. The old trabeculæ are represented by thick bands of rather dense fibrous tissue, enclosing a mesh of young connective tissue and endothelioid cells. In this mesh may be large numbers of blastomycetes free and intracellular. Young blood vessels may be numerous. In the cells which arise from the trabeculæ there is almost no phagocytosis.

Lungs. — In the earliest stage of blastomycetic infection single blastomycetes can be seen in the walls of the alveoli. Sometimes it is certain that such a blastomyces lies in the blood vessel in the alveolar wall, but as a rule it is impossible to say whether it lies in a blood vessel or in clefts between the cells of the alveolar wall.

At a slightly later stage, although the alveolar spaces persist, they are reduced in size by proliferation of the tissue of the alveolar wall. The character of the cells which produce this thickening is as in other proliferated areas, *i.e.*, connective tissue and endothelioid cells. In these early areas are numerous blastomycetes intracellular or free, sometimes with, often without, the gelatinous capsule. Occasionally the phagocytic cells are giant cells. There is no evidence of proliferation of the alveolar epithelium, and blastomycetes are not seen free in the alveolar spaces. Lymphoid and plasma cells and eosinophiles may be fairly numerous. The perivascular lymph spaces often are dilated, contain large numbers of lymphoid cells, granular detritus and endothelioid cells, and an occasional blastomyces.

As the proliferation increases the areas of new-formed

tissue enlarge and encroach upon the alveoli and practically obliterate them. The areas of proliferation may become very large. Sometimes in areas in which proliferation is well marked blastomycetes are necrotic or very few in number. Extreme and diffuse proliferation may practically obliterate the alveolar spaces over a large portion of an entire lobe.

In some lungs the alveoli between and adjacent to the proliferative areas show marked desquamative pneumonia. The alveoli are dilated, contain leucocytes, serum, fibrin, and blood, and often large numbers of desquamated alveolar epithelial cells.

In many cases, where there is no evidence of proliferation, large areas of the lung are atelectatic. In such areas no or very few blastomycetes may be found. In one case, in such an atelectatic area small circular nodules were seen, closely resembling tubercles. The nodules were small and circular, the centre was composed of fibroblasts and endothelioid cells, many of the latter being phagocytic. In such areas blastomycetes always were numerous. The periphery of such nodules showed an infiltration with lymphoid cells. In one guinea-pig, which lived five months, whose lungs showed no gross lesion, there were small areas composed of very dense fibrous tissue in which were small irregular areas of epithelial cells. These areas were like those seen in a chronic interstitial pneumonia, and represented lung scars in which were contracted alveoli with the epithelial cells pressed together. In these areas no blastomycetes were seen.

Liver. — As a rule the liver showed no sign of invasion by blastomycetes, except by direct extension to the surface from other organs, *e.g.*, abdominal scar, or adherent intestine. In the cases of direct extension on the surface of the liver were small nodules of proliferated tissue such as is seen in the other organs. In one case (Plimmer's organism) in the liver there were small nodules of newly formed cells always of small size. Most commonly these foci were in the vicinity of one of the large hepatic vessels. There was no evidence of proliferation of the liver epithelium, nor of phagocytosis by liver cells.

In one case (neoformans) in the liver of an animal which lived three months there were numerous areas of newly formed tissue, partly endothelioid cells, partly spindle-shaped connective tissue cells. These areas began about the interlobular vessels and extended toward the intralobular vessel. This new tissue included small groups of liver cells, which at times were unaltered, at times were necrotic. In the necrotic areas was marked infiltration with an acute inflammatory exudate. At times the endothelioid cells were phagocytic and enclosed lymphoid cells, but nowhere were blastomycetes seen. There was no sign of proliferation of the liver cells.

Spleen. — In the spleen the process begins always in the Malpighian follicles, sometimes at the periphery, sometimes at the centre of the follicle. In the very earliest stage an occasional free blastomyces is seen in the lymphoid tissue of a follicle. In a later stage many blastomycetes may be seen, generally free, with a gelatinous capsule lying in a mesh of young connective tissue and endothelioid cells. This proliferation may continue until all of the lymphoid tissue in a follicle is replaced by newly formed tissue. Some of the endothelioid cells may be phagocytic. Sometimes free blastomycetes are seen in the sinuses of the splenic pulp. At times in the proliferated follicles lymphoid and plasma cells are numerous, and occasionally eosinophiles are seen. Occasionally the phagocytic cells in the follicle are polynuclear (giant cells). As the process of proliferation extends, it may go beyond the limits of the follicles, and ultimately the greater portion of the spleen be involved.

Kidneys. — In the kidneys the process is almost entirely confined to the cortex, and generally shows the earliest lesions in the glomeruli. In the earliest stage one or two free blastomycetes can be seen in the tuft of vessels in the glomerulus. Rarely it can be demonstrated that the blastomyces lies in the lumen of a capillary, but usually it is impossible to tell whether it lies in clefts between cells or in the capillary. In a later stage areas of newly formed tissues are seen. These areas may lie between the kidney tubules

just beneath the capsule, or deeper in the kidney at the junction of cortex and pyramids, but never among the straight tubules. The process generally, but not always, appears to begin in a glomerulus. If it does begin in a glomerulus the capsule soon is filled with newly formed connective tissue and endothelioid cells in and among which are numerous blastomycetes. Or the process may begin between the kidney tubules and form similar areas between the tubules. At the periphery of such areas there are flattened tubules lined with cells whose nuclei do not stain and whose protoplasm is more or less necrotic. An occasional lymphoid cell may be seen in such areas, but the reaction of the tissue surrounding one of the newly formed nodules is surprisingly small. The tubules in close proximity to such a nodule may be absolutely unaltered. There is no evidence of proliferation or phagocytosis by the renal epithelium. As a rule there is no invasion of renal tubules, but rarely a tubule included in one of these areas may show blastomycetes in the lumen. Sometimes a blastomyces is seen free in the glomerular space. At times the proliferated areas attain a large size. Sometimes the proliferation of tissue is very slight, even about a considerable mass of blastomycetes. Sometimes it seems as if the proliferative process began in a perivascular lymph space. Very seldom a blastomyces can be seen in a cast in renal tubules.

Brain. — In four animals there were well-marked lesions in the central nervous system due to the blastomycetes. In the brain and cerebellum the process is confined to the pia and to the perivascular lymph spaces. The pia is greatly thickened by a new growth, partly of connective tissue, but chiefly of an enormous formation of endothelial cells. Sometimes there is marked new formation of blood vessels. The endothelial cells in the pia frequently are of large size and usually are phagocytic. Often they are polynuclear, and the nuclei often have a marked mural arrangement. Extracellular blastomycetes also may be very numerous. Lymphoid and plasma cells may be present in great numbers. In the brain substance may be very numerous areas of similar

proliferation with large numbers of blastomycetes. Such areas are approximately circular and invariably are confined to the perivascular lymph spaces. At the periphery of these areas are seen fibers of the neuroglia tissue, projecting into the area, often showing evidence of necrosis of the fibers as well as of the ganglion cells. Outside the proliferative areas is absolutely no evidence of infiltration with any form of leucocyte.

Exactly similar changes can be seen in the pia of the cerebellum.

In the pia of the spinal cord the lesions also are the same. There is no sign of invasion of the cord itself by the blastomycetes.

Epidermis. — In several of the subcutaneous nodules the process caused by the blastomycetes extended toward the surface so that the corium was more or less completely destroyed and the colonies came into contact with the epidermis. In these cases the deeper layers of the epidermis are pushed apart by the blastomycetes, while the cells between them are partly necrotic and disappear. There is no evidence of proliferation of or phagocytosis by the epidermis. On the contrary, the cells of the epidermis disappear in front of the advancing blastomycetes.

Conclusions. — Examinations of the results of the experience of other men and of the experiments just reported lead to the conclusions that

1. Certain blastomycetes can live and multiply in human and animal tissues, produce local lesions and metastases in the internal organs, *i.e.*, they are pathogenic.

2. The lesions produced in animals by spontaneous infection with blastomycetes are acute inflammations, abscesses or nodules of peculiar granulation tissue, and are not in the least analogous to cancers.

3. The lesions produced in human beings in cases of spontaneous infection with blastomycetes are acute inflammation (abscesses or ulcers) or proliferation of endothelium and connective tissue. At times a proliferation of the epidermis does occur, but is not due to the action of the blastomycetes,

but is secondary to the chronic inflammation of the underlying corium. This proliferation of epidermis is not analogous to the proliferation of epithelium seen in cancers, since no epithelial metastases occur.

4. Blastomycosis in human tissues is very rare.

5. The lesions produced in animals by experimental inoculation with blastomycetes are, with the exception of Sanfelice's "successful" cases, inflammations or nodules of peculiar granulation tissue. Sanfelice's cases are not conclusive in themselves, are in direct opposition to the results obtained by all other observers, and, even if true, are logically explained as concidences, and not as results.

6. Blastomycetes as a rule cause marked proliferation of tissue, and little infiltration with leucocytes, *i.e.*, their toxic powers are small.

7. Blastomycetes primarily extend along lymphatic clefts and vessels.

8. Rarely in human beings, more often in spontaneously infected animals, and often in experimental animals blastomycetes may be taken into the blood vessels, disseminated throughout the body, and produce a general infection and metastases.

9. The secondary nodules have the same general character, *i.e.*, a formation of granulation tissue, as the original nodules.

10. The morphology of the so-called "cancer bodies" is not identical with that of the blastomycetes.

11. Blastomycetes are not constantly present in human malignant tumors and cancers.

12. Even if blastomycetes do occur in human cancers they are not present in such numbers and in such a relation to the anatomical lesion as to justify the belief that they are the cause of the disease.

All of these facts lead to the ultimate conclusion that there is no evidence that blastomycetes have anything to do with the production of human cancers.

REFERENCES.

1. Virchow. Zur Entwickelungsgeschichte des Krebses. Virch. Arch., 1847, I.–94.

2. Busse. Uber parasitäre Zelleinschlüsse und ihre Züchtung. Centralbl. f. Bakt., Aug. 11, 1894. Vol. xvi., 175.

3. Busse. Ueber Saccharomycosis hominis. Virch. Arch., 1895, cxl., 23.

4. Busse. Experimentelle Untersuchungen über Saccharomycosis. Virch. Arch., 1896, cxliv., 360.

5. Busse. Die Hefen als Krankheitserreger. Berlin, 1897.

6. Sanfelice. Ueber eine für Thiere pathogene Sprosspilzart. Centralbl. f. Bakt., 1895, Jan., xvii., 113.

7. Sanfelice. Über einen neuen pathogenen Blastomyceten. Centralbl. f. Bakt., 1895, xviii., 521.

8. Sanfelice. Ueber die pathogene Wirkung der Sprosspilze. Centralbl. f. Bakt., 1895, May, xvii., 625.

Sanfelice. Ueber die pathogene Wirkung der Blastomyceten.

9. Sanfelice. Zeitsch. f. Hygiene, Part I., 1896, xxi., 32.

10. Sanfelice. Zeitsch. f. Hygiene, Part II., xxi., 394.

11. Sanfelice. Zeitsch. f. Hygiene, Part III., 1896, xxii., 171.

12. Sanfelice. Zeitsch. f. Hygiene, Part IV., xxvi., 298.

13. Sanfelice. Zeitsch. f. Hygiene, Part V., 1898, xxix, 463.

14. Plimmer, H. G. On the Etiology and Histology of Cancer. London Practitioner, 1899, lxii., 430.

15. Roncali. Die Blastomyceten in den Adeno-carcinomen des Ovariums. Centralbl. f. Bakt., 1895, xviii., 353.

16. Binaghi. Ueber das Vorkommen von Blastomyceten in den Epitheliomen und ihre parasitäre Bedeutung. Zeitsch., Hygiene. 1896, xxiii., 2.

17. Aievoli. Richerche sui Blastomiceti nei neoplasmi. Centralbl. f. Bakt., 1896, xx., 445.

18. Mafucci and Sirleo. Beobachtungen und Versuche uber einen pathogenen Blastomyceten bei Einschluss desselben in die Zellen der pathologischen Gewebe. Centralbl. f. Path. u. path. Anat., 1895, iv., 305.

19. Mafucci and Sirleo. Neuer Beitrag zur Pathologie eines Blastomyceten. Centralbl. f. Path. u. path. Anat., 1895, vi., 438.

20. Mafucci and Sirleo. Weitere experimentelle Untersuchungen über einen pathogenen Blastomyceten. Centralbl. f. Allg. Path. u. path. Anat.

21. Mafucci and Sirleo. Ueber die Blastomyceten als Infektionserrege bei bösartigen Tumoren. Zeitsch. f. Hygiene, 1898, xxvii., 1.

22. Corselli and Frisco. Pathogene Blastomyceten beim Menschen. Centralbl. f. Bakt., 1895, xviii., 368.

23. Leopold. Untersuchungen zur Aetiologie des Carcinoms und über die pathogenen Blastomyceten. Arch. f. Gynäk., 1900, lxi., 1.

24. Curtis. Contribution à l'etude de Saccharomycose humaine. Ann. Institute Pasteur, 1896, x., 448.

25. Gilchrist. A Case of Blastomycetic Dermatitis in Man. Johns Hopkins Hospital Reports, 1896, i., 270.

26. Ricketts. Oidiomycosis (Blastomycosis) of the Skin and its Fungi. Jour. Med. Research, 1901, vi., 377.

27. Fermi and Aruch. Ueber eine neue pathogene Hefeart. Centralbl. f. Bakt., 1895, xvii., p. 593.

28. Tokishige. Ueber pathogene Blastomyceten. Centralbl. f. Bakt., 1896, xix., 105.

29. Buschke. Ueber Hefemykosen bei Menschen und Tieren. Sammlung klinischer Vorträge, 1898, Heft. 218. Read October, 1897.

30. Buschke. Ueber Hautblastomykose Dtsche. Dermatolog. Gesellschaft. 6th Congress, 181, 1899.

31. Colpe. Hefezellen als Krankheitserreger im weiblichen Genitalkanal. Arch. f. Gynäk., xlvii., 1894.

32. Ernst. Ueber Nierenmykose und das gleichzeitige Vorkommen verschiedener Pilzformen bei Diabetes. Virch. Arch., cxxxvi., 1894.

33. Neumayer. Untersuchungen über die Wirkungen der verschiedenen Hefearten auf den thierischen und menschlichen Organismus. Arch. f. Hygiene, 1891, xii., 1.

34. Nesczadimenko. Zur Pathogenese der Blastomyceten. Centralbl. f. Bakt., 1 Abth., 1899, xxv., 55.

35. Rabinowitsch. Untersuchungen über pathogene Hefearten. Zeitsch. f. Hygiene, 1895, xxi., 11.

36. Foulerton. Pathogenic Action of Blastomycetes. Journ. Path. and Bact., May, 1899, vi., 37.

37. Petersen. Die Bedeutung der Hefepilze in der Pathologie. Münch. Med. Wochenschr., 1898.

38. Petersen and Exner. Ueber Hefepilze und Geschwulstbildung. Beiträge zur klinischen Chirurgie, 1899, xxv., 769.

39. Richardson. Report of Culture Experiments with Carcinomatous Tissue, 1899–1900. Journ. Bost. Soc. Med. Sci., v., No. 2.

40. Bonome. Sull' importanza dei blastomiceti nei tumori. Atti del R. Instituto Veneto di Scienze, 1898, ix. Cited by Sanfelice, Pt. v.

41. Sternberg. Ueber die Zelleinschlüsse in Carcinomen und ihre Bedeutung als Blastomyceten. Ziegler's Beiträge, 1899, xxv., 554.

42. Greenough. Presence of the So-called Plimmer Bodies in Carcinoma. 1900. Jour. Bost. Soc. Med. Sci., v., 34.

43. Nichols. First Annual Report of Work on the Etiology of Cancer. 1900. Jour. Bost. Soc. Med. Sci., v., 59.

44. De Meser. Vorkommen von Licopodium Sporum im Innern eines Carcinoma der Haut. Virch. Arch., clxiii., Heft. 1, January, 1901.

DESCRIPTION OF PLATES.

PLATE XXV.

FIG. 1. — Blastomyces, with gelatinous capsule, included in mononuclear phagocytic endothelial cell.

FIGS. 2, 3, 4, 5, 6. — Blastomycetes, gelatinous capsule not represented, as they appear in the tissues.

FIG. 7. — Portion of primary nodule after inoculation with Sanfelice's neoformans. Shows a bit of a connective tissue trabecula, with endothelioid cells, some of which are phagocytic, and free blastomycetes lying in capsular space.

FIG. 8. — Edge of abscess as seen in Figure 9, shows a bit of connective tissue trabecula, and a mass of endothelioid cells. No blastomycetes are seen.

All drawn with Zeiss comp. oc. 4; obj. 2 mm., apert. 1.30.

FIG. 9. — Ear of rabbit, inoculated in ear vein. Abscess formed, discharged, and closed. Shows dense connective tissue stroma surrounding a zone of endothelioid cells, which in turn enclose small abscesses. Zeiss comp. oc. 4; obj. 16 mm., apert. 0.30.

PLATE XXVI.

FIG. 1. — Portion of lung. Plimmer's organism. Alveolar walls thickened, and in newly formed tissue numerous blastomycetes. One blastomyces seen in a large blood vessel.

FIG. 2. — Portion of primary nodule. Plimmer's organism. Blastomycetes lie in capsular space between endothelioid cells. Blastomycetes show spine-like projections, due to contraction of gelatinous capsule.

FIG. 3. — Portion of lymph sinus in lymph node. Sanfelice's neoformans. Blastomycetes with gelatinous capsule in free endothelial cells.

FIG. 4. — Follicle of spleen. Plimmer's organism. Shows proliferation and blastomycetes in the middle of the follicle.

FIG. 5. — Secondary nodule in the kidney. Plimmer's organism. Proliferation of endothelioid cells, many blastomycetes with gelatinous capsule, and practically no inflammatory reaction.

FIG. 6. — Kidney nodule. Sanfelice's neoformans. More advanced than Figure 5, which began in a glomerulus. Glomerular tissue has entirely disappeared.

PLATE XXVII.

FIG. 1. — Testis of rabbit. Plimmer's organism. Shows slightly altered tubules, with blastomycetes and newly formed tissue between the tubules. 300 diameters.

FIG. 2. — Primary nodule. Sanfelice's neoformans. Blastomycetes and gelatinous capsule between endothelioid cells. One endothelioid cell is phagocytic. 1,110 diameters.

FIG. 3. — Primary nodule. Plimmer's organism. 1,110 diameters.

1

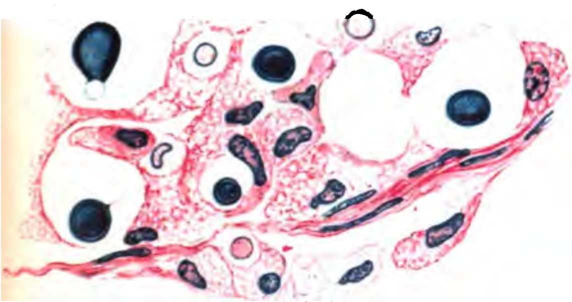
2

3

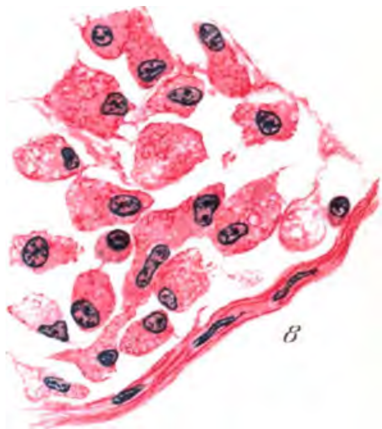
4

5

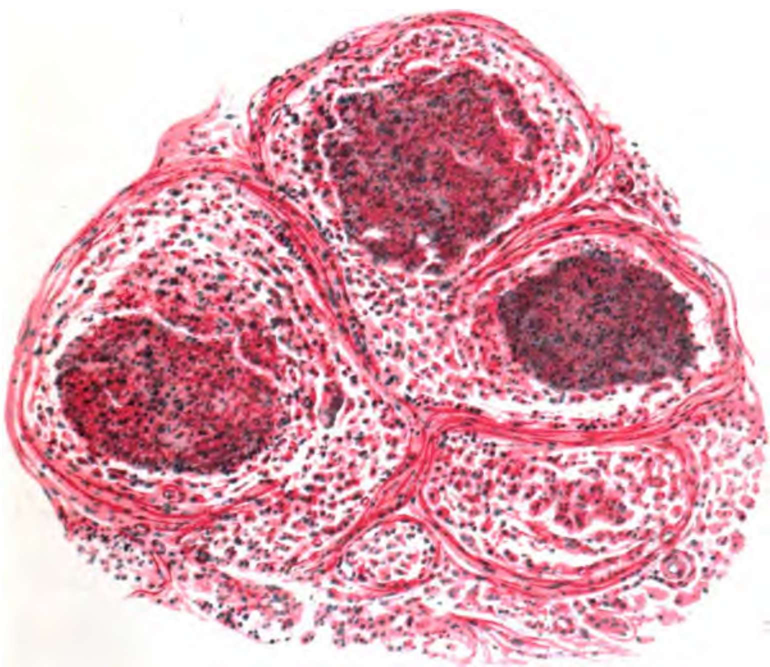
6



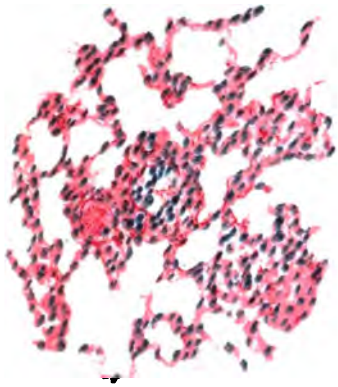
7



8

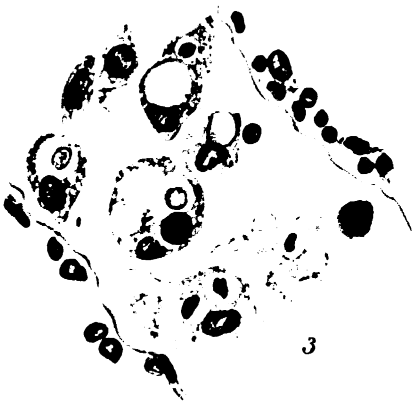


9

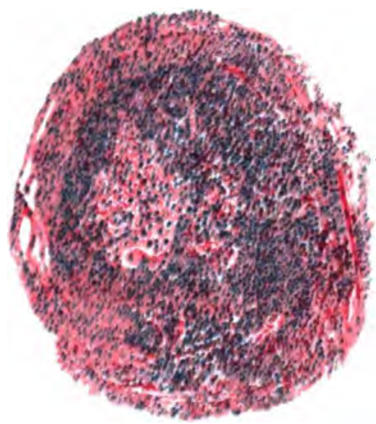


1

2

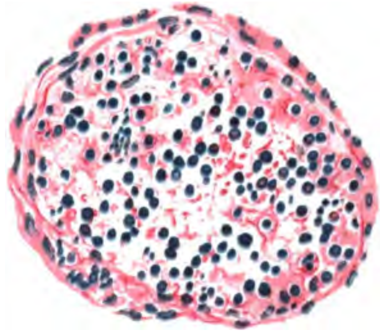


3

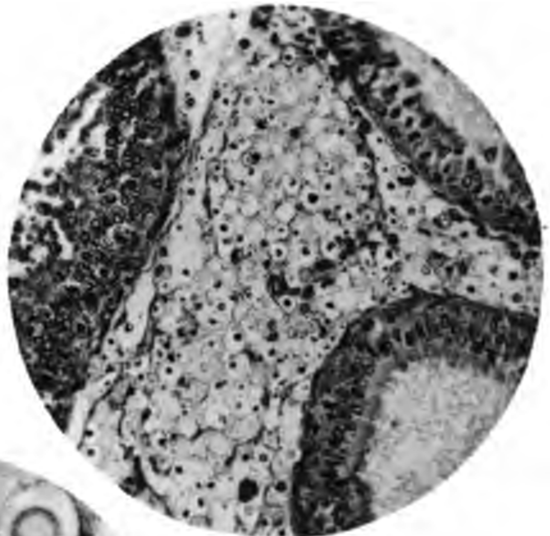


4

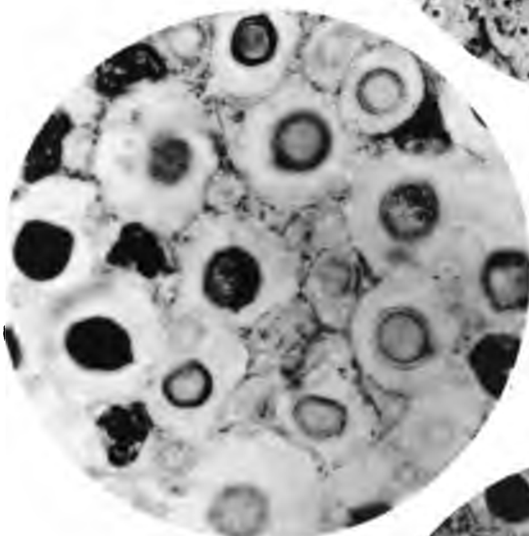
5



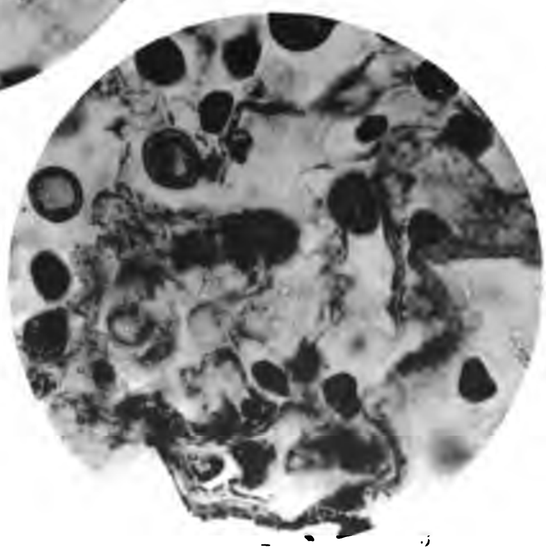
6



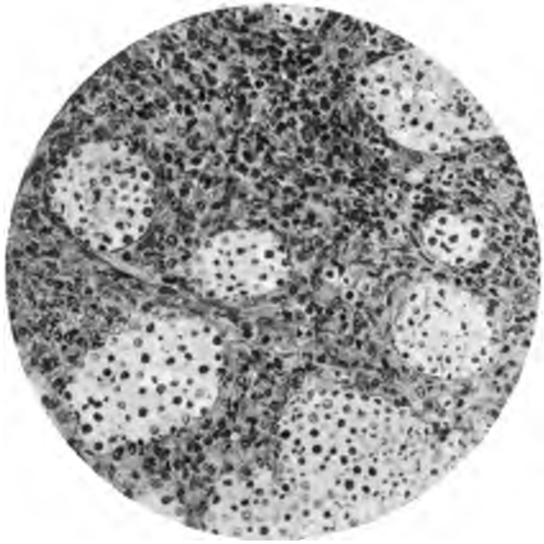
1



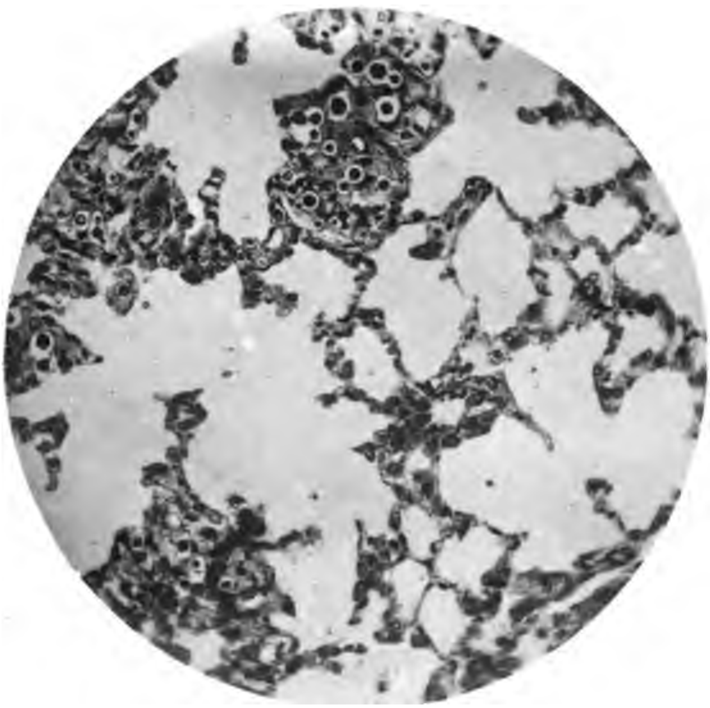
2



3



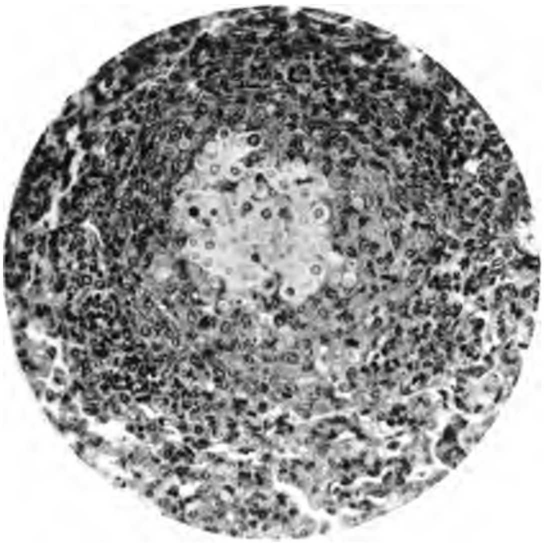
1



2

Journal of Medical Research.

Vol. VII. Plate XXIX.

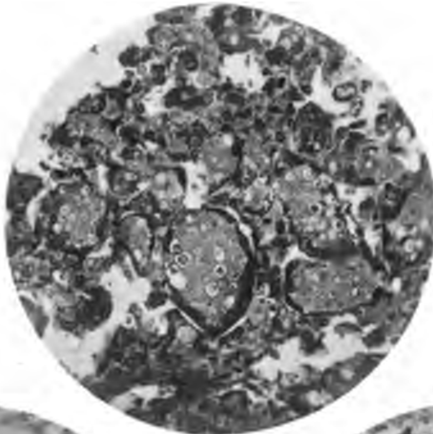


1

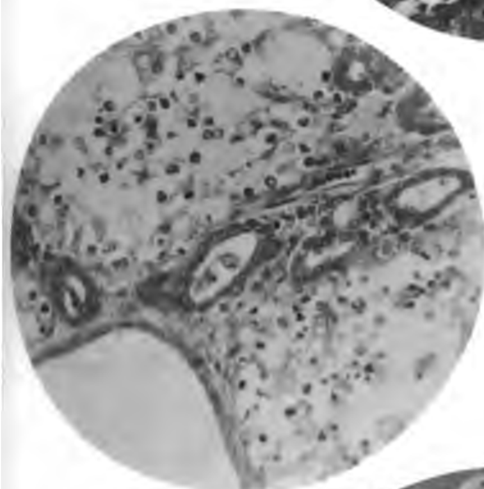
2

Blastomycetes and Cancer.

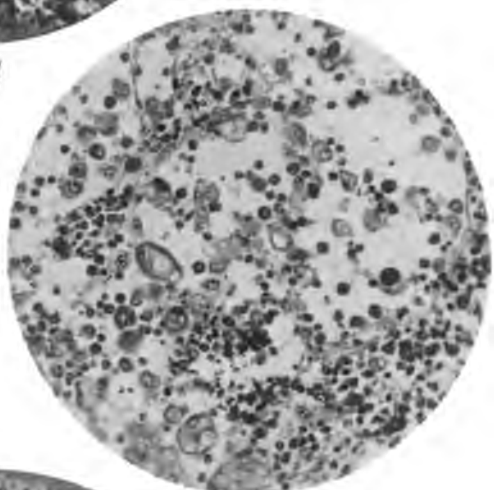
Nichols.



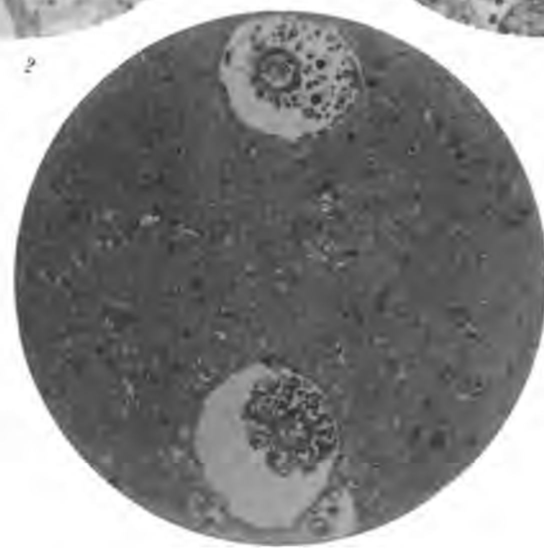
1



2



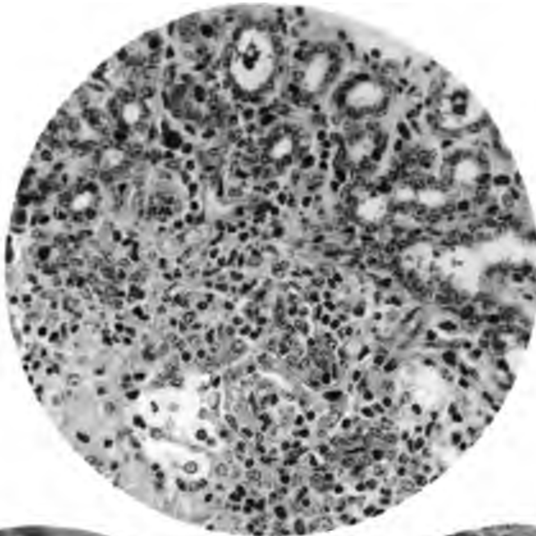
3



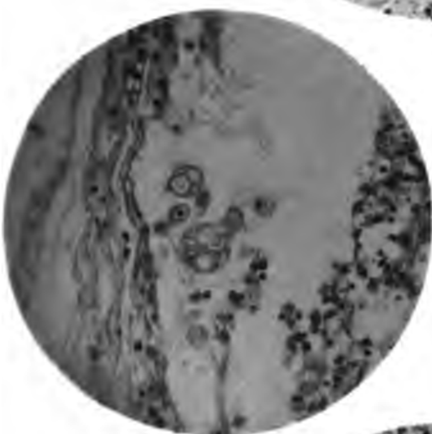
4

Nichols. Blastomycetes and Cancer.

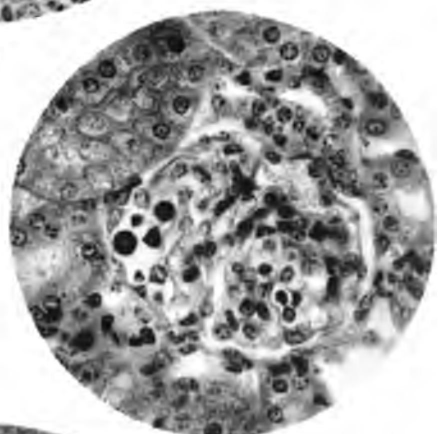
Heliotype Co., Boston.



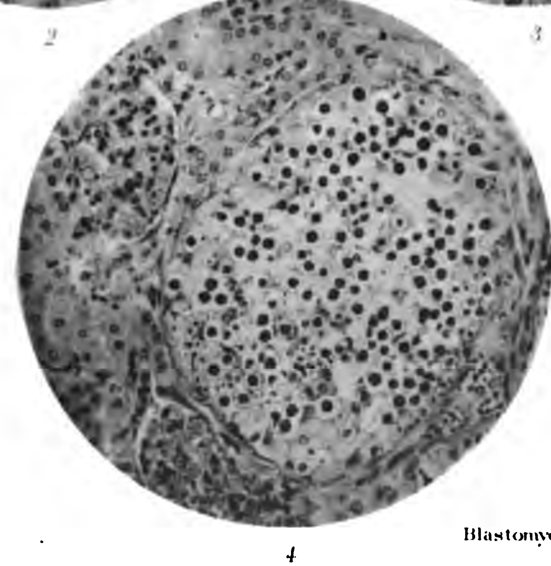
1



2



3



Nichols. Blastomycetes and Cancer.

4

PLATE XXVIII.

FIG. 1. — Lymph node. Sanfelice's neoformans. Late stage. Lymphoid cells chiefly destroyed. Trabeculæ greatly thickened. Light areas are remnants of sinuses and are filled with blastomycetes.

FIG. 2. — Lung. Sanfelice's neoformans. Alveolar walls in places much thickened, and contain numerous blastomycetes.

PLATE XXIX.

FIG. 1. — Blastomycetic "tubercle" in an atelectatic lung. Plimmer's organism. Blastomycetes in the centre, surrounded by a zone of endothelioid and connective tissue cells, with some lymphoid infiltration at the periphery.

FIG. 2. — Lymphoid follicle in the spleen. Sanfelice's neoformans. Lymphoid tissue largely destroyed, and replaced by a mesh of endothelioid and connective tissue cells. Blastomycetes free and in phagocytic cells. A few giant cells. 90 diameters.

PLATE XXX.

FIG. 1. — Endothelial cells and connective tissue in pia of brain. Plimmer's organism. Many blastomycetes, mostly in phagocytic giant cells.

FIG. 2. — Primary nodule of the breast. Sanfelice's neoformans. Glands widely separated, and practically unchanged. Newly formed tissue and blastomycetes between the glands.

FIG. 3. — Sinus in lymph node. Sanfelice's neoformans. Many free endothelial cells, many of which are phagocytic.

FIG. 4. — Blastomycetes and new tissue in the perivascular lymph space of cerebral blood vessels. Plimmer's organism.

PLATE XXXI.

FIG. 1. — Breast nodule, — primary, — in a case in which the nodule receded. Glands show but little change. Sanfelice's neoformans. A few blastomycetes with gelatinous capsule. Glands separated by rather dense fibrous tissue and endothelioid cells. Some infiltration with lymphoid cells.

FIG. 2. — Free endothelial cells in sinus of lymph node. Plimmer's organism. Many of endothelial cells are phagocytic.

FIG. 3. — Blastomycetes in the glomerulus of a kidney. Plimmer's organism. Very early stage and no evidence of cell proliferation. 350 diameters.

FIG. 4. — Large nodule in cortex of the kidney. Sanfelice's neoformans. Very little dense fibrous tissue at the periphery of nodule. Nodule consists chiefly of blastomycetes, and a few endothelioid cells. Adjacent tubules are flattened. Practically no infiltration with leucocytes or lymphoid cells. 220 diameters.

[Colored drawings by Miss Florence Byrnes.]

CELL INCLUSIONS IN CANCER AND IN NON-CANCEROUS TISSUE.

R. B. GREENOUGH, M.D.
(*From the Clinico-Pathological Laboratory of the Massachusetts General Hospital.*)

In the first annual report of the Cancer-Investigation Committee of the Harvard Medical School, in 1900, the writer reported the examination of a series of specimens of carcinoma with the following conclusions:

(1.) The appearances known as "Plimmer's bodies" were found in each of twenty-three cases of breast cancer.

(2.) They were more numerous in the periphery of the tumors and in the metastases.

(3.) They were not found in areas which had undergone even slight degeneration, whether before or after removal.

(4.) They were more numerous in the slow-growing carcinomata, and less frequently found in the rapidly growing ones.

(5.) They were more numerous in scirrhous than in medullary and adeno-carcinoma types of cancer.

(6.) They were not found in three cases of the epithelioma type (one of which was a typical Paget's disease of the breast).

(7.) They were present in one case of ovarian carcinoma, and absent in another case of general peritoneal cancer, of probable ovarian origin.

These conclusions were sufficiently suggestive to warrant the further investigation of the occurrence of these appearances in other tissues, and during the past year the writer has examined ninety-seven specimens of tissue, both normal and pathological, by the same methods of fixation and staining as were employed before, and with the following results.

The greater part of the material for this investigation was obtained from Dr. W. F. Whitney and Dr. J. H. Wright, of

the Massachusetts General Hospital, to whom the writer would here express his deep obligation, not only for the specimens, but also for many invaluable suggestions and much assistance. The material was cut into small pieces and placed at once in either Zenker's or Hermann's fluid, Flemming's solution, or Pianese's Osmic Acid and Chlorplatinate fixative. After twenty-four hours the tissues were washed, put through ascending alcohols and chloroform, and embedded in paraffin. Sections were stained after Plimmer's method with Iron-Hematoxylin and counterstained with Orange G. and Fuchsin, or with Bordeaux red. Some sections were stained after Pianese's special methods No. 3 A. and No. 3 B.[1] Where the nature of the tissue did not forbid, sections of two mikrons were obtained by the use of the Minot-Blake microtome, but occasionally sections of three or four mikrons were as thin as could be cut, owing to the density of the fibrous tissue.

The description given by Plimmer in his article in the

---

[1] PIANESE, ZIEGLER'S BEITRÄGE, SUPPLEMENT-HEFT NO. 1, JENA, 1896.

Hardening Method.

Chlorplatinate of soda 1% Aq. sol.	15 ccm.
Chromic acid, .25% Aq. sol.	5 ccm.
Hyperosmic acid, 2% Aq. sol.	5 ccm.
Formic acid C. P.	1 drop.

Staining Method.

No. 3 A.

Malachite green	1 gram.
Acid fuchsin	.40 gram.
Nigrosin	.10 gram.
Aq. dist.	50 ccm.
Alcohol sol. sat. with Acetate of Copper	50 ccm.

Use 20 drops of this solution to 10 cc. Aq dist.

Stain 24 hours. Decolorize with 1/8% Aq. sol. oxalic acid. Stains resting nuclei light red, protoplasm reddish yellow, mitosis green, chromatin (nuclein) green.

No. 3 B.

Malachite green	50. cg.
Acid fuchsin	10. cg.
Martius yellow	1. cg.
Aq. dist.	15. ccm.
Alcohol, 96%	50. ccm.

Stain without diluting one-half hour. Decolorize in absolute alcohol. Nuclei green, protoplasm rose, cancer bodies red and green.

"Practitioner," April, 1899, is as follows: "The parasites, as they most often occur, are round bodies of very diverse sizes, from .004 mm. to .04 mm., or even more, in diameter. There is a central portion, which I shall call here, for convenience, the nucleus, although there is nothing in this central portion in common with the biological nucleus, which is generally round, but which may be irregular in shape; around the nucleus is a layer of protoplasm, and outside this is a capsule. This nucleus differs in its micro-chemical reactions from the nucleus of the cell; it takes, with the Ehrlich-Biondi solution, a copper-red color; with thionin, a dark purple; and with (1) of the double hæmatoxylon stains (Iron hæmatoxylon, Orange G. and Fuchsin) it also takes a copper-red color, quite different from the red of either the protoplasm or the fibrous stroma; and with (2) (Bordeaux red) it takes a dark claret color, again darker than that of the protoplasm or stroma. . . . It is to be noted that the nucleus is practically refractory to hæmatoxylin used in the strongest manner possible, when this is done by the ordinary methods." Such appearances Plimmer states that he found in 1,130 out of 1,278 cases of cancer of all parts and of all varieties, 513 of which were cancer of the breast, and 346 cancer of the skin (including tongue and penis).

The fact that these characteristic appearances were found to be practically constant in cancer of the breast, but were absent in those cases of epithelioma which were examined in 1900, led to a more extensive examination of epitheliomata as a preliminary step in the work for 1901. Fifteen cases of epithelioma were examined from the following sources: Lip, nine; ear, two; cervix, one; vagina, one; hand, one; jaw, one. Of this number two only showed appearances which could in any way be regarded as even resembling Plimmer's bodies, and these two specimens, both epitheliomata of the lip, were remarkable for the extensive and irregular formation of Keratin which they presented, and the predominance of "Pearl" formation. The intra-cellular structures found in these two cases, moreover, were situated exclusively toward the central and older portion of the epithelial col-

umns, and showed in no case the rayed periphery of the typical glandular cancer inclusion.

Two cases of papilloma, one of the skin and one of the cervix, were also negative.

Three cases of sarcoma were examined, one of the kidney, one of the abdominal wall, and the third a metastasis of melanotic sarcoma in a lymph gland, and all failed to show any trace of cell-inclusions which could be likened to Plimmer's bodies.

Of tubercular tissues four specimens were examined, but with negative results; three were lymphatic glands, and the fourth a tubercular ulcer of the tongue.

Two cases of pneumonia and one case of sub-acute adenitis of a lymph gland were also absolutely negative.

Seven specimens of normal animal tissue were included among the sections examined during the year: the Fallopian tube of the cat, the intestine and renal tubules of the water bug, the breast and liver of the rabbit, and the testis, epididymis, and intestine of the guinea-pig. These specimens failed to show any structures resembling Plimmer's bodies, although in the guinea-pig testis the attempt was made to confirm the results of Borrel, who found a resemblance between "the attraction sphere" of mitosing cells and the classical cancer inclusion. Borrel's stain of sulph-indigotate of soda, however, was found to work better with human than with animal tissues and to its use in this connection reference will later be made.

Specimens of the normal human testis and of the placenta showed no evidence of cell inclusions, and a dermoid cyst of ovary and an adenoma of prostate were also negative.

Of the human intestine seven specimens were examined — three of the large intestine and four of the appendix. In three of the seven a striking appearance was noted which was thought to have a bearing upon the question of the origin and nature of cell-inclusions in glandular cancer. This appearance is in the nature of an artifact, but may apparently be produced after the use of any fixative and is in fact more probably a result of the subsequent treatment with

alcohol necessary to any method of paraffine imbedding than to the primary fixation. This appearance consists of the formation in or near the centre of the space in the gland cell in which the droplet of mucin is normally collected, of a darker staining area with irregular outline and surrounded by threads and fibres running toward the unstained periphery of the space. This central staining area occupying the space in which we know the mucin secreted by the gland cell is collected can only be regarded as a coagulation (or centripitalization as it has been called by Pianese) due to the solidification by fixing and hardening agents of a substance which was fluid and homogeneous during life. The staining of substances being as a rule in a proportion to their condensation, it is natural that such a coagulation should stain more deeply than the surrounding parts. The radiating fibres may be regarded as the points at the periphery which were more firmly attached than others and thus resisted the coagulating and shrinking force which tended to draw the whole mass toward the centre of the vacuole. In the intestine these central staining areas are undoubtedly of the character of mucin and take a diffuse stain with hæmatoxylin which prevents the detection of any chromatin particles in their constitution. Zimmermann, however, has succeeded in staining intestinal mucosa so as to bring out the presence in each of these mucin vacuoles of one or more chromatin particles, which he regarded as centrosomes. (Plate XXXII., Fig. 1.)

Four adenomatous tumors of the ovary and one papillary ovarian cyst with extension to the peritoneum were examined, but in only one of the adenomata could structures be found resembling the typical cell-inclusion, and in this tumor the inclusions were present only in papillary out-growths within the cyst wall. The malignant papilloma, with extension to the peritoneum, had undergone too much post-mortem change to show any other intracellular structure than the nucleus either in the epithelium or the connective tissue, but was of particular interest for this very reason, as it presented almost the duplicate of the appearances in the connective tissue of a similar tumor obtained in Rome by

Dr. Weis, regarded by Roncali and Sanfelice as undoubted blastomyces.

Attention was then directed to secreting gland tissues and three submaxillary salivary glands were examined, but without the discovery of any intracellular structures similar to cancer cell-inclusions. The phenomena of secretion as presented in the submaxillary gland were striking, and suggested strongly that the nucleus played a part in the production of the secreted substance. Three stages of the process could be distinguished: 1, acini distended, nuclei small with much chromatin and pushed to the outer limit of the cell, while the portion of the cell toward the lumen was filled with unstained droplets of secretion; 2, nuclei more vesicular, less chromatin than before, and the free border of the cell containing few discrete droplets of unstained substance; 3, acini shrunken, nuclei large and well netted with chromatin, protoplasm less in amount, homogeneous and finely granular. In the walls of some of the larger ducts appearances similar to inclusions could be made out in one case, but in the secreting tubules no such appearances could be detected.

Four specimens of thyroid tissue, all adenomata, but clinically not of the malignant type, showed evidence of secretion in abundance. Where large cysts were present their contents were found to take the characteristic colloid stain, but a zone of unstained or faintly stained substance surrounds each colloid mass, lying between it and the secreting cells of the cyst wall. In this lightly stained area empty vacuoles and occasionally a vacuole with a central darker area can be clearly seen. Where the tubules are small the lumen is found to contain similar vacuoles, and the suggestion is obvious that the colloid metamorphosis is one which the secretion undergoes after its elaboration rather than that it is secreted as colloid from the beginning. In the thyroid, as in the submaxillary gland, the difference between adjacent nuclei in the amount of chromatin they contain was notable. No centrosomes could be detected in the masses of secretion.

Thirty specimens of non-malignant breast tissue were obtained during the year — Acute, Sub-acute, and Chronic

Mastitis, Adenoma, Adeno-fibroma, and Myxo-fibroma. Specimens of the normal breast at rest and in lactation were also included in the series. The results of the examination of these thirty specimens were of considerable interest, inasmuch as nineteen of them showed intracellular structures which bore the closest resemblance to the inclusions seen in gland cancer.

The normal breast at rest presents no intra-cellular appearances which can be likened to the cancer cell-inclusion. In the lactating breast intra-cellular structures are abundant, chiefly of a fatty nature, staining with osmic acid and appearing as droplets in the portion of the cell toward the lumen of the duct. These droplets frequently show a central area which takes the protoplasm stain more vigorously than the periphery. The free contents of the lumen show the same structure, being composed largely of fatty droplets of all sizes, but showing a certain amount of granular material taking the protoplasmic stain in which small areas of chromatin can be detected. The nuclei of the secreting cells show numerous variations in size and in chromatin content, which are analogous to the variations in the submaxillary gland and can be summarized briefly as being in such proportion that the more intensely stained and compact the nucleus the more secretion in the cell. Mitosis was not observed.

In the resting gland, although no intra-cellular structures can be detected, the lumen of the tubule presents an appearance which has been thought to have a bearing on the question of the nature of the cancer inclusion. The contents of the lumen, a homogeneous granular material, taking the protoplasmic or acid stain, never fills out the entire space around which the gland cells are arranged, but invariably shows a concentration generally irregular and eccentric in some part of this area, leaving an empty space about it. This appearance of the contents of the lumen varies in size with the size of the tubule, but in the smaller tubes presents exactly the appearance of the intra-cellular cancer inclusion. The presumption of concentration or centripetalization of the previously fluid contents of the lumen is so strong as to suggest

that a similar process might well produce similar appearances in the retained secretion in the cancer cell.

Two cases of acute mastitis (breast abscess) in the lactating breast were examined, one being completely broken down, the other showing in places the normal appearance of the breast in lactation as described above.

Of two cases of sub-acute mastitis, both due to pyogenic bacteria, one was extensively degenerated, and was negative; the other showed vacuoles of secretion in the gland cells, some of which contained the characteristic central staining area of cancer inclusions.

Eleven cases of chronic mastitis of the declining breast, with cyst-formation, but with no evidence of malignant change, were examined, and in nine of these, characteristic and typical cell-inclusions were found. Since the pathology of chronic mastitis is not worked out to allow of any well recognized definition of its histological appearances, it may be stated that for the purposes of this investigation only such specimens were included as showed:

(1.) Chronic inflammatory changes in the connective tissue; including increase in the amount of this tissue and round-cell infiltration about the tubules.

(2.) Atrophy of certain gland-tubules, and dilatation into cysts of other tubules, with corresponding proliferation of the gland epithelium in the cystic regions.

(3.) Absence of any epithelial growth beyond the limits of the basement membrane of the gland duct.

(4.) Absence of any marked irregularity in the size and shape of the gland cells.

(5.) Absence of mitosis of any marked degree of frequency and of any degree of irregularity.

Two cases were thrown out as not coming up to the standard above set forth, and will be discussed later with the cases of breast cancer.

In the proliferated epithelium lining the cystic cavities, evidences of secretion are numerous, and are accompanied by the same changes in the appearance of the nuclei to which attention has been called above. The gland epithe-

lium takes on at times a high columnar form; although all degrees of height between this and the normal nearly cuboidal cell of the resting breast on the one hand, and the small flattened atrophic cell of a distended cyst on the other, occur as transition forms. The nucleus of the high columnar cell is ordinarily situated near the basement membrane, and betweeen it and the free surface of the cell there appear in the protoplasm a number of granules of variable size, which stain with the acid stains. Toward the free surface in many cells a vacuole appears, sometimes with but more often without a central darker staining area. Occasionally in the centre of the vacuole, which may attain any size up to that of the nucleus, there can be seen both acid and basic staining material — that is, a larger protoplasmic staining mass with one or more small spots which retain the hematoxylin, and present the staining reactions of chromatin. (Plate XXXII., Fig 2. Plate XXXIII., Fig. 1.) Where the epithelial cells are atrophied, no such appearances can be detected; but where proliferation occurs in the smaller tubules, particularly when the lumen has been encroached upon, and the outlet for the secretion, as it were, obstructed, these vacuole structures, with their central staining masses, become larger and more prominent, and are practically constant in their situation in the cell, lying between the nucleus and the free border of the cell toward the lumen of the tube. These structures are the exact counterpart in their staining properties and their intra-cellular position to the typical cancer inclusion, and only in their size, which rarely exceeds that of the nucleus, and is more often about one-third to one-half of the diameter of the nucleus, do they fail to duplicate the cancer inclusions in every way. The cancer inclusion, it may be said, is also generally smaller than the nucleus, although in some cases exceeding it in size. (Plate XXXIII., Fig. 3. Plate XXXIV., Fig. 2).

Of the two cases entered as chronic mastitis which did not show these appearances, one was partly degenerated, due to post-mortem changes, and showed vacuoles only, without central staining areas, — the other was an atrophic breast, the

epithelium of which was so flattened and shrunken as to show no intra-cellular structures whatever.

Of adeno-fibromata of the breast there were eight cases, and in five of these typical secretory phenomena were observed of the same nature as in chronic mastitis, and with the same resemblance to the cancer inclusion. The epithelial cells and their nuclei were, as a rule, smaller in the fibromata than in mastitis, and the same held true of the intra-cellular structures, which, however, bore about the same relation to the size of the nucleus as in other tissues. (Plate XXXII., Fig. 3. Plate XXXIV., Fig 1.) Of the three cases in which no such bodies could be found, one was markedly degenerated, and the other two showed much atrophy of the epithelium with corresponding loss of protoplasm and protoplasmic structure. Where present, however, the protoplasmic central area and the occasional chromatin spot were as marked as in mastitis. In one of these adeno-fibromata the attempt to stain with Borrel's sulph-indigotate of soda was successful in demonstrating the intense carmine spot (chromatin) in the midst of the blue protoplasmic central area, which was characteristic of the archoplasm or "attraction sphere" in the guinea-pig's testis, when this special stain was used.

One case of myxo-fibroma of the breast may be included here as being the type of malignant change in pre-existent adeno-fibromata, and in this tumor also the same intracellular structures could be demonstrated; not, however, in the myxomatous connective tissue, but in the breast epithelium lining the cystic cavities.

Four adenomata of the breast were obtained during the year — two papillary and two only cystic. In three of these tumors changes as above described were present in the epithelial cells and could not be distinguished from cancer inclusions except by the tissue in which they occurred. In one of these cases in particular (of which Plate XXXIV., Fig. 2, is a drawing and Plate XXXIII., Fig. 3, a photograph), the situation of the bodies at the free border of the cell toward the lumen of the tubule was most striking; and their

internal structure of acid and basic staining material most convincingly demonstrated.

Eight cases of cancer of the breast were included in the list of the year's specimens; some being entered for comparison, others being found to be cancer only after section and careful examination. Two of these cancer cases were early cases in which the process was beginning in a breast which elsewhere presented only the appearances of chronic mastitis, and in both cases typical inclusions could be demonstrated not only in the cancerous but in the non-cancerous portions. In the other six cases of more advanced cancer, inclusions were found in every case.

As an accessory to the work of this year, and in the light of this year's results, some of last year's specimens have been again examined and a number of facts noted which may be stated briefly here.

(1.) The size of the typical cancer cell-inclusion. In any specimen of cancer in which they are at all numerous an under limit of size is very hard to place; smaller and smaller bodies may be found, each giving the typical form and reaction to staining agents, but diminishing in size until of such minute proportions as to be distinguished with difficulty from any other granule in the cell protoplasm. (Plate XXXV., Fig. 1.)

(2.) Staining reactions. Sections stained with iron-hematoxylin invariably present in some of the bodies a larger or smaller mass of deeply stained substance, presumably chromatin, in the midst of the protoplasmic staining central area. This was attributed last year to over-staining with hematoxylin, but such proves not to be the case, since they resist decolorization at times even more than the nucleus. They must be regarded as chromatin or some kindred substance in the make-up of the cell-inclusion. Plimmer's plates show the same phenomenon, although the statement in the text, above quoted, that the bodies do not take the nuclear stain, is in contradiction to the testimony of the figures. (Plate XXXV., Fig. 2.)

(3.) Situation. In cancer tissue which has departed from

the type of the gland structure from which it arose, no landmarks are left to determine in what portion of the cell the inclusion is situated, and it is precisely in these forms of cancer that the inclusions are largest and most numerous. In adeno-carcinoma, however, where the tendency still remains for the cells to group themselves about an open space and repeat the structure of the original gland tissue, inclusions can be detected. In such cases the inclusions are smaller than in advanced cancer and more like the appearance in non-cancerous tissue. Their situation, too, is like that of the inclusions in non-cancerous gland tissue; that is, they lie between the nucleus and the free border of the cell toward the lumen of the duct. In many cases an irregularity in the line of the cell margin forming the wall of the gland duct suggests strongly that these structures, after attaining sufficient size to distend the cell, have been extruded into the free space formed by the lumen of the gland. Were this the case the absence of the larger forms of inclusion in adeno-carcinoma and their presence in cancer of the scirrhous type would be readily explained by the fact that where no lumen exists the secretion cannot escape, but is retained in the cell to undergo further growth or degeneration according to the vitality of the cell. This theory of retained secretion has been advanced by Pianese and by Galeotti and would seem to be supported by the results of this investigation.

Many different parasitic theories for cancer etiology have been built upon the microscopical appearances of cancer tissue, but it remained for Plimmer, in 1899, to make such startling claims for the constant presence of typical cell-inclusions in cancer of all varieties as to cause the former inclusions to be rechristened with his own name. Plimmer found typical bodies of constant morphology in cases of cancer of all varieties. These assertions of Plimmer's, together with the vigorous claims of Sanfelice and the believers in the blastomyces theory, gave new life to the old controversy, and have been followed by a renewal of activity in the field of cancer investigation. Among the prominent writers on cancer etiology who have opposed the parasite doctrine in

the past are Rippert, Hansemann, Fabre-Domergue, Pianese, Eberth, and more recently Borrel. In general the inclusions in cancer have been attributed to a number of different sources. The following are from Hansemann: (1) Degenerations of cells and anomalous secretion. (2) Phagocytosis. (3) Invagination. (4) Abortive and pathological mitosis. (5) Special organs of the cell, Archoplasm or Paranucleus. (6) Extra-cellular hyaline drops (Russell's). (7) Cancer cells themselves.

In all probability it is true that phagocytes, invaginations, anomalous mitotic figures, and degenerations of the cell and of the nucleus have been mistaken for parasites and described as such by Korotneff, Pfeiffer, Eisen, and many other writers. The fact remains, however, that there are a certain number of characteristic and typical inclusions in gland cancer which are figured, together with other non-characteristic figures, by all the writers who wish to prove a parasitic origin of cancer. Degenerations of cells and of nuclei occur in every kind of tissue and are found in cancer and sarcoma as well as in tissues which are not malignant. The typical inclusion of gland cancer is a structure which is not ordinarily seen in other tissues, and its interpretation by the opponents of the parasite theory has been the subject of considerable diversity of opinion. Pianese, whose monograph is by far the most elaborate of all the works we have on cancer histology, used special staining methods of his own, and withal was unable to state whether the typical inclusion of glandular cancer, as figured in his plates, was a product of the secretion of the cell or of the degeneration of the nucleus. Most pathologists have agreed in attributing a nuclear origin to these inclusions, and Eberth and Borrel are among the few who have relieved the nucleus of all responsibility for their origin and made them dependent upon the activity of the protoplasm of the cell itself.

From the specimens examined in the last two years it is evident that typical inclusions are practically constant in glandular cancer and extremely rare or absent in epithelioma. This fact at once throws doubt upon the assertion that the

inclusions are due to nuclear degenerations. It is inconceivable that nuclei which grow and divide and degenerate in the same fashion in squamous epithelium and in gland tissue should show so marked and individual a difference in their forms of degeneration in the corresponding cancer growth. Far more readily can it be believed that the cell-inclusion, which is so constant in cancer of glandular origin, is associated in some way with that function which is common to all gland tissue, namely, secretion. Cancer of squamous-celled origin produces keratin pearls in its atypical growth; adeno-carcinoma of the large intestine shows more or less typically the secretion of mucin in its goblet cells; and malignant adenoma of the thyroid may give rise to colloid material even in remote metastases.

The thyroid and the mucous glands of the large intestine differ from the mammary gland, however, in one particular. Their function is practically constant, while that of the breast is intermittent if not wholly latent. Many observers, to be sure, have seen in the frequent and extensive fatty degeneration of breast cancer a connection with the function of lactation. Extensive fatty degeneration, however, is hardly more characteristic of one form of cancer than of another, and is equalled if not surpassed by many sarcomata and fibromyomata and readily explained by lack of adequate blood supply.

As a fact, however, while the functional breast produces milk, the resting gland is not entirely devoid of secretory function. A section of a resting breast shows glandular epithelium surrounding an open space, the lumen of the gland. In this space there is a collection of granular homogeneous material, taking the acid or protoplasmic stain, and evidently the result of the secretory activity and degeneration of the surrounding cells. In the stage of decline of the mammary gland, near the menopause, when the fibrous tissue is increased and chronic mastitis so frequently occurs, small cysts are found with greenish viscid contents. This too is evidence of the secretory activity of the non-lactating breast.

The histology of secretion has been studied in many glands, but in the mammary gland, unfortunately, a unanimity of opinion among different observers does not seem to exist, and attention has been mainly directed to the gland in lactation, so that the resting gland has been somewhat neglected.

It is generally admitted that during pregnancy active growth in the breast gland occurs; the acini increase in number and size, karyokinetic figures are frequently seen. Some writers (Steinhaus, Duclert) were able to distinguish a peculiar phenomenon of growth in the breast during pregnancy, in that the mitoses occurred in the gland epithelium perpendicular to the basement membrane and resulted in an increase in length of the gland tubule; whereas later, after lactation was established, the mitoses were parallel with the basement membrane and merely supplied the loss of cells by wear and tear.

Immediately after labor, Duclert found globules of colloid substance, rounded masses of different sizes, in the protoplasm of the gland epithelium; but after thirty-six hours had elapsed and lactation was established, these colloid masses gave way to the familiar droplets of fat of the milk producing gland. Here again is evidence of the secretion of a substance other than fat.

During lactation the minute phenomena of secretion are not so clearly defined, some writers (Haidenhaim, Nissen, Steinhaus) believing that a degeneration of the whole cell, with destruction of its nucleus, occurs with each contribution to the secretion, and others (Bizzozero, Vassale, Benda, Ottolenghi) claiming for the breast epithelium, as established in the sub-maxillary and other glands, the capacity of elaborating and discharging the secretion repeatedly, until by natural processes the individual cell dies and is replaced. There is certainly a marked absence of mitotic figures in the lactating breast, and no evidence of a direct nuclear division has been advanced; so that it is hard to believe that almost the entire secreting surface of the gland is being renewed at the enormously rapid rate which would

be necessary if each cell perished in contributing its droplet to the common stock. It seems certain, however, that a few mitoses, less than during the growth of the gland in pregnancy, do occur, and the more rational view to accept is the one that admits of repeated secretory activity for each cell, with its ultimate exhaustion and renewal.

The histologists who favor the complete degeneration and constant renewal theory are influenced by finding traces of supposed nuclear degenerations and of chromatin in the secretion, and attribute to this destruction the well-known nuclein of the milk.

In the sub-maxillary gland the secretion is alternately elaborated and extruded by the living cell, and the minute histology has been more accurately studied. Clumps of deeply staining filaments or rods are found in the protoplasm near the nucleus. Between and about these filaments the secretion collects in drops which fuse together and ultimately burst the cell membrane and are extruded (Solger). These observations were confirmed by Garnier and Hammar, and the name of "ergastoplasm" applied to this differentiated portion of protoplasm. Prenant regards this ergastoplasm of the secreting cell as the analogue of the kinoplasm, or attraction sphere of the dividing cell in which the chromatin staining centrosome appears at the poles of the achromatic spindle; and Bizzozero and Vassale think that the centrosome and ergastoplasm are the source of the chromatin in the nuclear-like figures described by Nissen and Haidenhain, and that the entire process is one of cell activity, beginning with centrosome and ergastoplasm and ending in the droplet secretion. This contention is further supported by the elaborate researches of Zimmermann, who found centrosomes associated with secretion in the lachrymal and salivary glands and the mucin-forming glands of the stomach and intestine. The situation of the droplet of secretion in all these observations is practically constant, lying between the nucleus and the free margin of the cell.

To recapitulate briefly, then: the phenomenon of secretion in glandular epithelium is associated with a definite differen-

tiated portion of the cell protoplasm with which a chromatin body, the centrosome, is probably involved.

The chief reason upon which Pianese, Hansemann, and Fahre-Domergue and others base their contention that the cancer inclusion is a form of nuclear degeneration is because in a large number of these typical inclusions a certain larger or smaller portion of the central mass will take the basic chromatin stain. This appears very plainly in the plates of Plimmer's article, and was noted repeatedly in the specimens examined last year, as has been stated above. Specimens stained with Pianese's method, 3 B, show this peculiarity most clearly. The histology of secretion, however, provides an explanation for the presence of this chromatin.

The nuclei of cancer cells in a given specimen may vary in size to a remarkable degree; the cell inclusions, however, far exceed them in this respect. In one portion of a specimen they may be found well defined, but so small that four or five occupy the space of a single nucleus, while in another part they will reach a size equal to twice that of the usual cancer cell. No degeneration can well make a structure of a given size both larger and smaller by the same process, but a droplet of secretion, starting as a minute particle and growing by fusion with other similar particles, may attain any size so long as rupture of the cell-membrane is prevented by the pressure of other structures on all its sides. Under normal conditions the secretion of a gland cell is extruded into the free space of the gland lumen when it attains a size sufficient to rupture the enveloping cell wall. In cancer, however, as suggested by Pianese, and in some other pathological conditions of the breast, enough proliferation of the epithelium occurs to obliterate the gland lumen, there is no space into which the secretion can be discharged, and it remains in the cell and there increases in size until the vitality of the cell is impaired and the whole structure undergoes degeneration.

The large numbers of cell-inclusions in gland cancer of the slow-growing types, and the comparative infrequency with which they are found in the rapidly growing carcino-

mata, suggest that an extreme degree of departure from the normal type of tissue has occurred, and that the higher function of secretion has been lost in the increase of capacity for growth. This characteristic of cancer we are familiar with in other forms of growth, as in epithelioma and the sarcomata. In this connection it may be said that in only one instance to our knowledge has a typical cell-inclusion been found in a mitosing cell.

The theory advanced by Borrel, which attributes the cancer inclusion to a metamorphosis of the archoplasm, is so near to the present hypothesis as to be practically the same. The archoplasm, however, is a phenomenon of cell-division, and we have found that inclusions are more abundant in the slow-growing types of gland cancer than in the more rapid growths where mitoses and cell divisions are comparatively frequent. The phenomena of cell division, moreover, are not confined to gland epithelium, but occur in all tissues, and should present the same appearances in sarcoma and in epithelioma as in gland cancer.

The phenomenon of secretion, on the other hand, is accompanied by a differentiated portion of the cell protoplasm, the ergastoplasm, which is in every way analogous to the archoplasm of the dividing cell, and it is to the ergastoplasm that the writer would attribute the typical inclusions of gland cancer. The primary cause, however, must be traced back beyond the ergastoplasm, and will be found in the inherent tendency of the gland cell to fulfil its function, the production of secretion.

That the inclusions of cancer were due to secretion has been maintained by numerous writers (Durante, Pianese, Hansemann). The proof of this theory, however, has not been fully established. It has been the object of the writer of this paper to contribute the results of his own observations, and to analyze the observations of the writers on the histology of normal and of cancer tissues, with the hope of establishing the secretion theory on a more secure foundation.

Conclusions.

1. Cell inclusions of a constant type are found in practically all cases of cancer of the mammary gland.

2. They are also found in non-cancerous disease of the mammary gland.

3. They are not found in epithelioma or sarcoma.

4. Their appearance, staining reactions, and situation in the cell are such as to justify the hypothesis that they are the result of the secretory activity of the epithelial cell.

5. There is no cause for attributing a parasitic origin to their appearance.

BIBLIOGRAPHY.

Cancer Histology.

Adami. British Med. Journal, 1901, March 16.
Borrel. Annales Institute Pasteur, Paris, xv., No. 2, 1901.
Brosch. Virchow Archiv, Vol. 162, Heft 1.
Demeser. Virchow Archiv, 1901, March.
Fabre Domergue. Presse Medicale, Paris, 1896, p. 157.
Galleotti and Lustig. Zeigler's Beiträge, xiv., 228.
Hansemann. Berlin Klinische Wochenschrift, 1894, No. 1.
Hansemann. "Bösartige Geschwülste," Hirschwald, Berlin, 1897.
Hauser. Centralblatt fur Allg. Path. und path. Anat., Jena, Vol. ix., pp. 221 and 867.
Lohmer. Zeigler's Beiträge, Vol. 28, 1900, p. 372.
Petersen and Exner. Beiträge zur Klin. Chirurgie, Vol. xxv., Heft 3.
Petersen. Centralblatt fur Chirurgie, xxviii., 1901, p. 4.
Pianese. Zeigler's Beiträge, 1896. Supplement-heft 1.
Pichler. Wiener Klinische Wochenschrift, 1900, No. 4.
Rippert. "Pathologische Histologie," Cohen, Bonn, 1896.
Strobe. Zeigler's Beiträge, Vol. xi., 1892, p. 1.
Virchow. Virchow's Archiv, Vol. 3, 1849.
Völcker. Deutsche Medicinische Wochenschrift, 1901, July 25.
Bosquest. Compte rendue Soc. de Biologie, Paris, 1897, 10 S., iv., 1023.
Eisen. Medical Record, N.Y., 1900, July 7.
Foa. Centralblatt für Bakter., 1892, xii., 6, p. 185.
von Leyden. Zeitschrift für Klin. Med., xliii, p. 1.
Podwyssotsky and Sawtschenko. Centralblatt für Bakter., 1892, xi., p. 493.
Schueller. Centralblatt für Chirurg., 1901, No. 33. (Review.)
Sjöbring. Fortschritte der Med., 1890, viii., p. 529.
Sjöbring. Centralblatt für Chirurg., 1901, xxviii, No. 29.
Soudakewitsch. Annales Institute Pasteur, 1892, vi., pp. 145 and 545.
Thoma. Fortschritte der Med., 1889, June 1.

Histology of Secretion.

Benda. Dermatol. Zeitschrift, '93–94, p. 94.
Bizzozero and Ottolenghi. Anatomische Ergebnisse, Vol. 9. Meckel and Bonne, Abtheil 2, 1899.
Bizzozero and Vassale. Virchow Archiv, 1887, No. 110, p. 155.
Duclert. Thèse Montpelier, 1893. [Quoted by Prenant.]
Steinhaus. Archiv Anat. und Phys., Suppl. Band, 1892.
Eberth and Müller. Zeitsch. für wiss. Zool. Suppl. Heft liii., 92.
Garnier. Bibliographie anatomique, 1899, Th. 7
Henry. Archives d'anat. Microscop (Ranvier), iii., 229.
Haidenhain. Archiv für Microscop. Anat., xliii., 1894, p. 423.
Kollesnikow. Virchow Archiv, 70, 531, 1877.
Maximow. Archiv für Mikr. Anat., 1901, 58.
Meves. Archiv für Mikr. Anat., 1901, 329.
Nissen. Archiv für Mikr. Anat., 1886, 337.
Prenant. Journal de l'Anatomie, Vol. 34, p. 657; Vol. 35, p. 52–167–408.
Rauber. Schmidt's Jahrbuch, Vol. 182, p. 7, 1879.
Solger. Anatom. Anzeiger, Bd. ix., 13 and 14.
Szabo. Archiv für Anat. und Physiolog. Anat., 1896, p. 352.
Von Unger. Virchow Archiv, Bd. 151, 1898.
Zimmermann. Verhand. der Anat. Gesellschaft, 1894, Vol. viii. 244.
Zimmermann. Archiv für Mikr. Anat., Vol. 52, p. 552.

DESCRIPTION OF PLATES.

The sections from which these photographs and drawings were made were stained with Iron-Hematoxylin and counterstained with Orange G. and Fuchsin, or with Bordeaux Red. The photographs were prepared by Mr. L. S. Brown, of the Massachusetts General Hospital, and the drawings were done by Miss Florence Byrnes, of the Harvard Medical School.

Plate XXXII.

Fig. 1. — Mucus Gland from Appendix. Centripetalization of mucin in vacuoles (x 250).

Fig. 2. — Chronic Mastitis. Wall of cyst, showing nuclei and secretion bodies in the cell (x 700).

Fig. 3. — Adeno-Fibroma of breast. Single layer of cells in a gland tubule. Secretion bodies lying between nucleus and lumen (x 700). See Plate XXXIV., Fig. 1.

Fig. 4. — Adeno-Fibroma of breast. Gland tubule with double layer of cells. Secretion body lying between nucleus and lumen (x 700).

Plate XXXIII.

Fig. 1. Cyst-Adenoma of breast. Cyst wall with high columnar Epithelium, in which vacuoles of secretion are present (x 125).

Fig. 2. — Cyst-Adenoma of breast. Tubule with proliferated epithelium in which a typical inclusion is present (x 500).

FIG. 3. — Cyst-Adenoma of breast. Small tubule with single layer of proliferated cells. Lumen almost obliterated. Inclusions lying between nucleus and lumen (x 500). (See Plate XXXIV., Fig. 2.)

PLATE XXXIV.

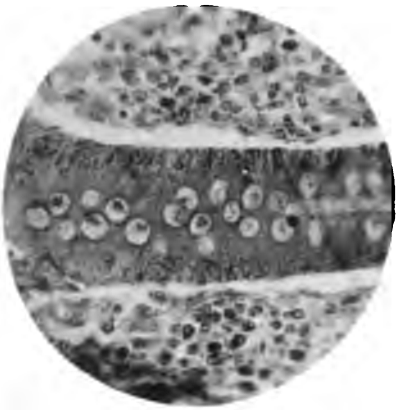
FIG. 1. — Adeno-Fibroma of breast. Intra-cellular secretion bodies, showing protoplasmic staining central area, between nucleus and lumen. Irregular outline of lumen due to extrusion of similar bodies from other cells (x 700). (See Plate XXXII., Fig. 3.)

FIG. 2. — Cyst-Adenoma of breast. Small tubule with single layer of cells. Intracellular bodies, taking protoplasmic stain, and showing central chromatin spots and rayed periphery between nucleus and lumen (x 700). (See Plate XXXIII., Fig. 3.)

PLATE XXXV.

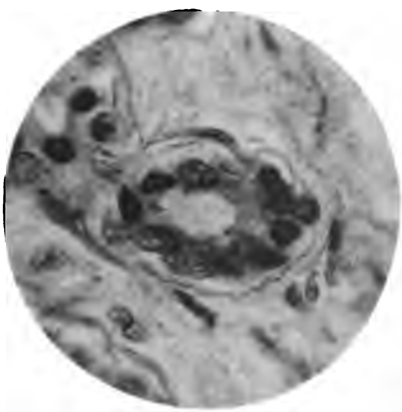
FIG. 1. — Adeno-Carcinoma of breast. Gland lumen in middle of column of cancer cells. Irregular outline of lumen. Inclusions with protoplasmic staining central area and chromatin spots lying between nucleus and lumen. Note small size of inclusions (x 700).

FIG. 2.—Adeno-Carcinoma of breast. Another portion of same specimen as Fig. 1. More advanced carcinoma. Absence of gland lumen. Typical inclusions' with protoplasmic staining central area, rayed periphery, and chromatin spots. Note larger size of inclusions than in Fig. 1 (x 700).

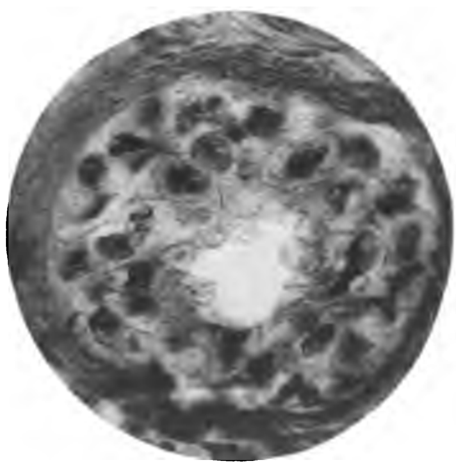


1

2



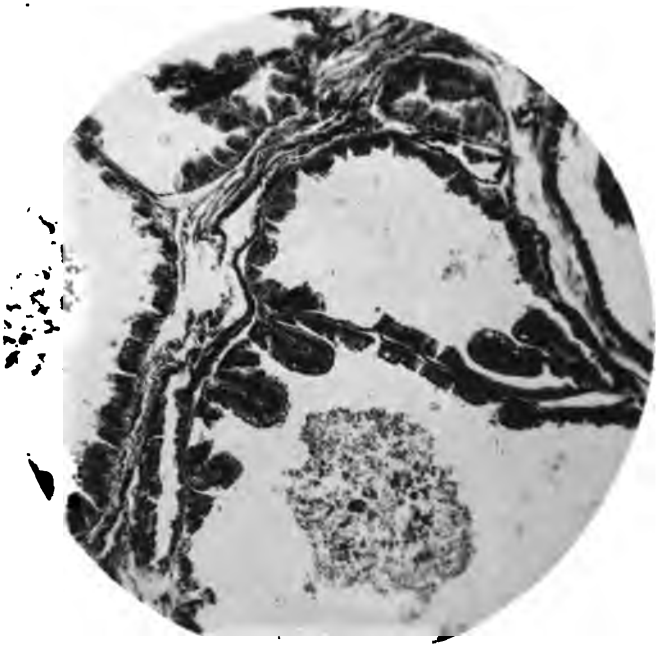
3



4

Greenough. Cell – inclusions.

Heliotype Co. Boston.



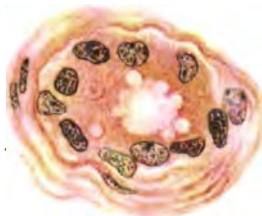
1

2

3

Greenough. Cell - inclusions.

Heliotype Co. Boston.



1

2

Greenough

Cell inclusions in Cancer

3

4

SUMMARY.

EDWARD H. NICHOLS.

It has been claimed by the adherents of the theory of the parasitic origin of cancer that

(1.) A proliferation of epithelial cells analogous to the lesions seen in cancerous tumors can be produced by certain well-known protozoa (nodules caused by the coccidium oviforme);

(2.) Certain skin lesions characterized by epithelial cell proliferation are due to the action of a so-called protozoon (molluscum contagiosum);

(3.) Blastomycetes are constantly present in human cancers and are the cause of the lesion;

(4.) By experimental inoculations of animals with "blastomycetes," true epithelial or cancerous nodules can be produced;

(5.) Finally, the well-known endocellular bodies seen in the protoplasm of cancer cells have a definite morphology, are "parasites," and the cause of cancer.

It has been the object of the investigators, the results of whose work appear in the preceding pages, to study each of these questions.

As a result of the lines of work pursued by them (under the direction of the Cancer Commission) during the past year it is concluded that

(1.) The lesion produced by the coccidium oviforme is essentially a process of chronic inflammation and is not analogous to the lesion seen in cancer.

(2.) The lesion in molluscum contagiosum is characterized by certain changes in the epidermis, is not due to the action of a protozoon, and is not analogous to cancer.

(3.) The so-called "blastomycetes" ("saccharomycetes") of Sanfelice and Plimmer are torulæ.

(4.) The lesions produced by these "blastomycetes" (torulæ) are essentially nodules of peculiar granulation tissue, are not cancerous, nor in any sense true "tumors."

(5.) Blastomycetes are not constantly present in human cancers.

(6.) The peculiar bodies seen in the protoplasm of cancer cells are not parasites, nor the cause of the lesions, but probably are in part at least atypical stages of the process of secretion by glandular epithelium.

THE

Journal of Medical Research.

(New Series, Vol. II.)

Vol. VII. MAY, 1902. No. 4.

THE ANALOGIES BETWEEN PLIMMER'S BODIES AND CERTAIN STRUCTURES FOUND NORMALLY IN THE CYTOPLASM.[1]

E. R. LeCount.

(*From the Pathological Laboratory of Rush Medical College, Chicago.*)

It is well known that methods involving the fixation of protoplasm by compounds of mercury are responsible in a large measure for recent additions to our knowledge of its structure. The demonstration of centrosomes and the variations in the surrounding archoplasmic structures have led, as is so generally the rule with advancements in science, to a confusion of nomenclature somewhat appalling to him who chooses to invade modern cytologic publications. The cells of the human body, and, generally speaking, animal cells, in which centrosomes and the surrounding diversely named formations in the archoplasm have not been described and depicted, are an ever decreasing number.

On the other hand, those investigators of the finer histologic details of tumors, and especially carcinoma and its cells, though not abandoning the osmium compounds, have also recently made extensive use of sublimate mixtures. Although Plimmer recommended the latter, he found Hermann's solution more useful. Greenough, however, found Zenker's fluid preferable to Hermann's, and certainly the former depends to a considerable degree upon mercury for its virtues. Many others have selected sublimate solutions not only for their splendid powers of fixation, but also for the ease with which subsequent treatment with aniline dyes is possible. I can especially commend aqueous solutions of mer-

[1] Read March 28, 1902, at the Second Annual Meeting of the American Association of Pathologists and Bacteriologists, at Cleveland, Ohio.

curic chloride to any one who wishes to examine carcinoma cells for Plimmer's bodies. It is therefore a noteworthy observation that while advancement in general cytologic studies — zoölogy, botany, and embryology — has been toward a more thorough understanding of the extranuclear architecture of resting and dividing cells, many, and especially the recent studies of the finer structure of the cells of malignant tumors have had as their purpose the demonstration of parasites — parasites that are also extranuclear, and the technical methods employed by these two sets of investigators have been to a large extent similar.

The history of progress in those sciences pertaining to medicine abounds in instances of improper interpretation of observations dependent solely upon the failure of investigators in different domains to take account of developments in other fields than their own. The resulting stumbling, hesitant, and gradual manner in which actual advances are finally made needs no comment.

The examination of carcinoma cells has been conducted from various points of view. The extensive work of Pianese[1] resulted in the establishment of various forms of nuclear and cellular degeneration and aberrations of mitosis. Other investigators have confined their labors to the demonstration of complex and abnormal forms of division, and the work by Hansemann, " Studien über die Specificität, Altruismus und Anaplasie der Zellen," is an oft-cited example of extreme and radical views issuing from prolonged labor in a constricted field. The recent work of Sternberg[2] upon the reactions of carcinoma cells with Gram's staining method apparently had the definite object of refuting statements of certain Italian observers relative to blastomycetes in the tumor cells. Certainly the work of Plimmer[3] upon malignant tumors had for its dominating purpose the demonstration of the bodies that

---

[1] Beitrag zur Histologie und Aetiologie des Carcinoms. First supplement to Ziegler's Beiträge, Jena, 1896.

[2] Ueber die Zelleinschlüsse in Carcinomen und ihre Deutung als Blastomyceten. Ziegler's Beiträge, 1899, xxv, 554.

[3] On the Etiology and Histology of Cancer, with special reference to recent work on the subject. Practitioner, 1899, lxii, 430.

bear his name, since during six years he succeeded in finding them in twelve hundred and seventy-eight "cancers."

The influence of the Boveri-Van Beneden theory, that the centrosome constitutes the "dynamic center" of the cell from which proceed all those impulses leading to cell division, doubtless caused the early investigators of malignant tumors to observe and search for centrosomes in dividing cells only. Certainly where centrosomes are mentioned in their writings it generally is in connection with cell division, notwithstanding that such structures have long been considered as permanent and essential parts of cells.

The backward swing of dissent from the dynamic theory mentioned has resulted from repeated observations of centrosomes and archoplasmic structures in resting cells; from the growing opinion that division of the nucleus is not so dependent upon division of the centrosome as formerly believed; and from the discovery of some cells in which present methods have failed to reveal centrosomes and which nevertheless divide by karyokinesis.

It would, therefore, seem an appropriate time to study carcinoma cells for centrosomes, attraction spheres, ecto- and ento-spheres, and the various other cytoplasmic formations described in normal cells. Such a study would seem warranted by the general failure of those supporters of the parasitic etiology of carcinoma, whose evidence is largely based on histologic studies, to even consider the existence of such elements in tumor cells, and by the further fact that there are certain analogies between the archoplasmic elements of normal cells and Plimmer's bodies.

The fact that such analogies occur was impressed upon me by a search for Plimmer's bodies in the axillary lymph glands removed in operations upon the mammary gland for carcinoma.

The structures originally described by Ruffer and Walker in 1893[1] and by subsequent workers, by Gaylord,[2] Nichols

[1] On Some Parasitic Protozoa found in Cancerous Tumors. Journal of Pathology and Bacteriology, 1893, i, 198.

[2] The Protozoon of Cancer. The American Journal of Medical Sciences, 1901, cxxi, 503.

and Greenough[1] in this country, and which are so well known as Plimmer's bodies, were found present without any difficulty in all the glands that contained epithelial cells. The lymph glands were obtained from seven cases and Plimmer's bodies were found in glands of six. In the remaining case no glands were obtained that contained epithelial cells. The points of similarity between these bodies and the archoplasmic structures of normal cells, a likeness that has been indicated by Borrel,[2] led me to stop further search in the regional metastatic growths for this "cancer parasite." The analogies are not simply those of morphology and position, but also of affinity for dyes. Stains that have been found especially applicable in the study of centrosomes have also found a field of usefulness in the demonstration of Plimmer's bodies.

One of the characteristics of Plimmer's bodies is that the central body of the parasite resists nuclear dyes; to use Plimmer's words, it is "refractory to hematoxylin used in the strongest manner possible."[3] It might be claimed that here is a radical difference between the nucleus of the parasite and the centrosome; on the other hand, although many cytologists claim that the centrosome invariably stains with the iron-hematoxylin method, Heidenhain[4] himself is by no means willing to concede this opinion.

Borrel, who used carbol-magenta with heat, and a counterstain of picro-indigo-carmine, controlled his method by the use of guinea-pigs' testicles; he found that the parasites stained with the same dyes as did the centrosomes of spermatogenic cells.

I have found the same bodies staining red with Borrel's method, and a fainter red with the methods advised by Plimmer. I have also found other hyaline bodies that often stain red with Borrel's method, like fuchsinophile bodies. Such

---

1 First Annual Report of Work on the Etiology of Cancer. Journal of Boston Society of Medical Science, 1900, v, 34.

2 Les Théories parasitares du Cancer. Annales de l'Institut Pasteur, 1901, xv, 49.

3 Loc. cit., p. 440.

4 Ueber die Microcentren mehrkerniger Riesenzellen sowie über die Centralkörperfrage in Allgemeinen. Morph. Arbeiten, 1897, vii, 247 (see foot-note).

hyaline structures bear less resemblance to centrosomes, and are not considered as characteristic Plimmer's bodies; they have also been noticed by Nichols.

A detailed description of Plimmer's bodies as they occur in the lymph glands would not add materially to existing records. Typical bodies possessed of single or double and distinct capsules with a more faintly staining zone between the capsule and so-called nucleus were readily found. Many others located in the same position as regards the elements of the epithelial cell correspond in all respects, except that the capsules were not as sharply defined. The rayed appearance of the periphery, although occasionally present, was, on the whole, rare. With Borrel's method, the encapsulating zone stained more readily than with the hematoxylin and iron method. Mallory's modification of the latter process of staining proved as useful as the original valuable technic devised by Heidenhain. Two central bodies of a single parasite were often found, and sometimes they appeared flattened against one another like a large biscuit-shaped diplococcus. Although usually round, oval and kidney-shaped central bodies were also encountered. Irregularities in form of the so-called nucleus of the parasite, were frequently met with in perfectly regular and circular formations. Two or more parasites were often seen in a single cell, although more than one was the exception. I have never seen a Plimmer's body in such a position that it could be safely stated that it was extracellular. As is well known, carcinoma cells are large cells, and in serial sections a single dividing nucleus often appears in two or three or more sections; therefore apparently extracellular parasites might belong to cells, a large part of which were to be found in other sections; nor were Plimmer's bodies seen in other than epithelial cells.

As regards the position of these bodies in the carcinoma cells, they usually lie close to the nucleus; in fact, as a rule, the periphery of the parasite is in contact with the nuclear membrane. Very often the Plimmer's body, where single and regular, lies in a slight incurvation of the nuclear membrane. Occasionally single Plimmer's bodies were found at

some distance from the nucleus, but more often this location obtained in cells that contained two or more parasites. In no instances did the glands show inflammatory changes.

It will be seen from the foregoing that there are analogies between Plimmer's bodies and the archoplasmic elements of normal cells, not only in their discovery by similar technical methods, but also in their position;[1] then, too, the sporulation or multiplication of the cancer parasite, by budding, is comparable to the division of the centrosome by simple cleavage. The archoplasmic structures described by Heidenhain in the giant-cells of a mesenteric lymph gland of a young rabbit that died with a diarrhea contained many (in some cells, forty to fifty) central bodies that are very comparable to forms described as Plimmer's bodies.[2] The tissue was fixed by Heidenhain[3] with a solution of mercuric chloride, and the pathological "microcentra," as Heidenhain calls the groups of centrosomes, were found accidentally when the tissue was stained with the Biondi-Ehrlich mixture. Afterward Heidenhain used rubin as a counter-stain with the iron-hematoxylin method.

The rayed periphery sometimes observed in Plimmer's bodies reminds one forcibly that the astral fibers of the archoplasm converge at the margins of the centrosphere. Centrosomes with radiating spheres, as before stated, have been described in many resting cells; although not many records of this kind concern cells of the human body, the observation of such formations in resting cells of so many lowly as well as more highly developed forms of animal life would apparently presage their general occurrence in human tissues.

Among the reports that effected the disestablishment of certain other so-called cancer parasites, there occur references and illustrations which in the light of present knowl-

[1] Cytomechanische Studien. Archiv für Entwicklungsmechanik der Organismen, 1895, i, 496.

[2] It may be contended, since the gland was diseased, that the structures described by Heidenhain were indeed parasites; granting this, the similarity between parasites and "microcentra" must be admitted, if Heidenhain, who has contributed so liberally to cytologic literature, was led into error.

[3] Loc. cit.

edge without doubt had to do with centrosomes and attraction spheres. Thus Ohlmacher[1] has pictured a centrosome with radiating fibers that he refers to as a "fuchsinophile" body, and Pianese,[2] in one of the beautiful figures that accompany his extensive work, has described as circumscribed hyaline degeneration of the cytoplasm a body that he likens to the parasites described by Soudakewitsch, and to those that Plimmer and Ruffer were unable to find in dividing cells. The cell represented by Pianese is in process of a division that he describes as abortive mitosis. The location, limitation, and staining reactions[3] are those of centrosome and archoplasm. I have met with many similar structures in the lymph glands. Galeotti[4] has made similar observations, and although both of the last-named observers recognize the structures described as identical with Plimmer's bodies, and although in both instances the cells are represented in mitosis, they not only failed to liken them to attraction spheres and centrosomes, but also neglected to describe or account for these formations so essential to karyokinesis.[5]

Borrel[6] has shown the analogy between the descriptions and illustrations of the cancer parasites in the work of Savtchenko[7] and the evolution of the centrosome and archoplasm in the spermatogenic cells of guinea-pigs' testicles. Doubtless a more thorough search through the publications which led to the final rejection of Russell's bodies and cell inclusions as parasites would reveal other records of bodies or formations that at present would be accepted as furnishing certain analogies to centrosome and centrosphere.

It may be contended in opposition to the view that Plim-

[1] A Brief Résumé of the Carcinoma Organism Question. Chicago Medical Recorder, 1892, iii, 466.

[2] Loc. cit., Plate II., Fig. 7.

[3] Pianese's method III a, that includes the use of acid fuchsin by which the centrosomes are stained red, was used.

[4] Beitrag zum Studium des Chromatins in den Epithelzellen der Carcinome. Zeigler's Beiträge, 1893, xiv, 249. Plate XIII., Fig. 20.

[5] Pianese (loc. cit., p. 89), it is true, remarks: "Die sogenannte Kernwand ist verschwunden, und man bemerkt keine Andeutung von Attraktionssphären, oder achromatischer Spindel."

[6] Borrel, loc. cit.

[7] Bibliotheca Medica, 1895, Abth. D, Heft. iv.

mer's bodies are normal or altered structures belonging to the cells containing them, that under normal conditions centrosomes are much smaller than Plimmer's bodies. In answer to this is the observation of Lustig and Galeotti,[1] that the centrosomes they found in carcinoma cells were much larger than the accounts of other investigators had led them to expect, and also the statement of Hansemann[2] that they are large (*unförmig gross*).

It is, however, true that the nuclei, so-called, of the Plimmer's bodies are generally much larger than the centrosomes of dividing carcinoma cells. But on the other hand it will be remembered that a typical Plimmer's body would include not only the central granule, but also the surrounding attraction sphere. Borrel has presumed that an atypical evolution of the archoplasm occurs in carcinoma cells and that the cancer parasites described are the results of such changes.

Since practically all the references to centrosomes in these cells are contained in studies of cell division, and since the work was done before the use of the sublimate method of fixation, it will be necessary, as has been before stated, to apply to the study of carcinoma cells the methods used in the demonstration of the archoplasmic structures in normal cells, before the conclusions of Borrel are established.[3] Heidenhain[4] in his classical studies of leucocytes showed that in them the nuclei and archoplasmic structures disappear last in processes of degeneration.

The predominance of Plimmer's bodies in the margins of tumors, in the growing parts, is as much in favor of an archoplasmic as a parasitic origin and furnishes another analogy; it also, perhaps, controverts Borrel's view that the bodies in question represent products of degeneration.

Among cytologists, Watasé[5] has suggested that the centro-

---

[1] Cytologische Studien über pathologische Gewebe. Ziegler's Beiträge, 1893, xiv, 225.

[2] Die Mikroscopische Diagnose der bösartigen Geschwülste, Berlin, 1897, p. 68.

[3] Borrel used Flemming's solution as a fixative.

[4] Ueber die Centralkörpergruppe in den Lymphocyten der Saugetiere während der Zellenruhe und der Zellteilung. Verhandl. der Anat. Gesellsch., 1893, 54–68.

[5] Origin of the Centrosome. Biological Lectures delivered at the Marine Biological Laboratory of Wood's Holl in the summer session of 1894, Boston, 1896, p. 273.

some is a differentiated microsome; others have contended[1] that either the nucleus or cytoplasm may give origin to the centrosome. It appears in cells about to undergo division, and in some cells it disappears when this is completed. Therefore, like Plimmer's bodies, centrosomes might not of necessity be present in all cells, yet like these so-called parasites they might be found in cells about to divide, which, however, contained resting nuclei.

A further analogy would perhaps be indicated by the observation of Nichols and Greenough[2] that some of the best examples of Plimmer's bodies were found in the more slowly growing carcinomas and that some of these contained large numbers; for if cell division was prolonged, there might be more cells in the tumor that would show such evidences of proliferation in the cytoplasm, as the appearance of archoplasmic spheres and centrosomes.

Under the heading "Les Maladiés â Sporozoaires," Bosc[3] has recently described bodies in the epithelial cells of the lesions of sheep-pox (*clavelêe*), smallpox, and vaccinia that he claims are identical with Plimmer's bodies. The technic used was largely sublimate fixation and staining with Biondi-Ehrlich's fluid or simple solution of aniline dyes.

Although this work awaits confirmation, at first glance its importance would seem rather to contradict the specificity of Plimmer's bodies for carcinoma than to establish the existence of a sporozoal group of diseases.

In conclusion, I believe that we can safely state that there are analogies between Plimmer's bodies in cancer cells and certain structures in the cytoplasm of normal cells, and that we are warranted in requiring enthusiastic advocates of the parasitic theory of carcinoma, who use Plimmer's bodies as a support for their views, to exclude or account for the centro-

---

1 The Cell in Development and Inheritance, by E. B. Wilson, 1900, p. 309, New York.

2 Loc. cit.

3 Les Maladiés à Sporozoaires: La variole, la vaccine, la clavelée (variole du mouton), le cancer. Arch. de Méd. exp. et d'Anat. path., 1901, xiii, 253.

some and the enveloping variously named formations of the archoplasm, in carcinoma cells.

I thankfully acknowledge my indebtedness to Professor Hektoen for many suggestions and his valued counsel, to the late Christian Fenger for a large part of the fresh material, and to Mr. T. Greig for drawings.

DESCRIPTION OF PLATES ACCOMPANYING DR. LECOUNT'S ARTICLE.

Plate XXXVI.

1. Copy of illustration of centrosome and archoplasmic structures accompanying an article by v. Lenhossék, "Centrosom und Sphäre in den Spinalganglienzellen des Frosches," Arch. für mik. Anat. u. Ent., 1895, xlvi, 345. (Fig. 13, Plate XVI. Large cell, 42 μ.)

2. Copy of an illustration accompanying Pianese's work (Plate II., Fig. 7). A cell is represented in mitosis, but the centrosomes and attraction spheres are undescribed, whereas a Plimmer's body is shown.

3. Copy of an illustration accompanying the article of Galeotti (Ziegler's Beiträge, 1893, xiv, Plate XIII., Fig. 20). He describes the cell as being in abortive mitosis, but fails to account for the centrosome or centrosomes.

(4–9. Plimmer's bodies from the axillary lymph glands in cases of carcinoma of the mammary gland.)

4. Plimmer's body: the similarity between the dividing nucleus of the Plimmer's body and a segmenting centrosome is very striking.

5. Plimmer's body: the position in an incurvation of the nucleus is that usually occupied by archoplasmic structures. A semilunar space around the body is often encountered and probably is an artefact. Central body deep red, surrounding body of parasite (or centrosphere), deep green (Borrel's method).

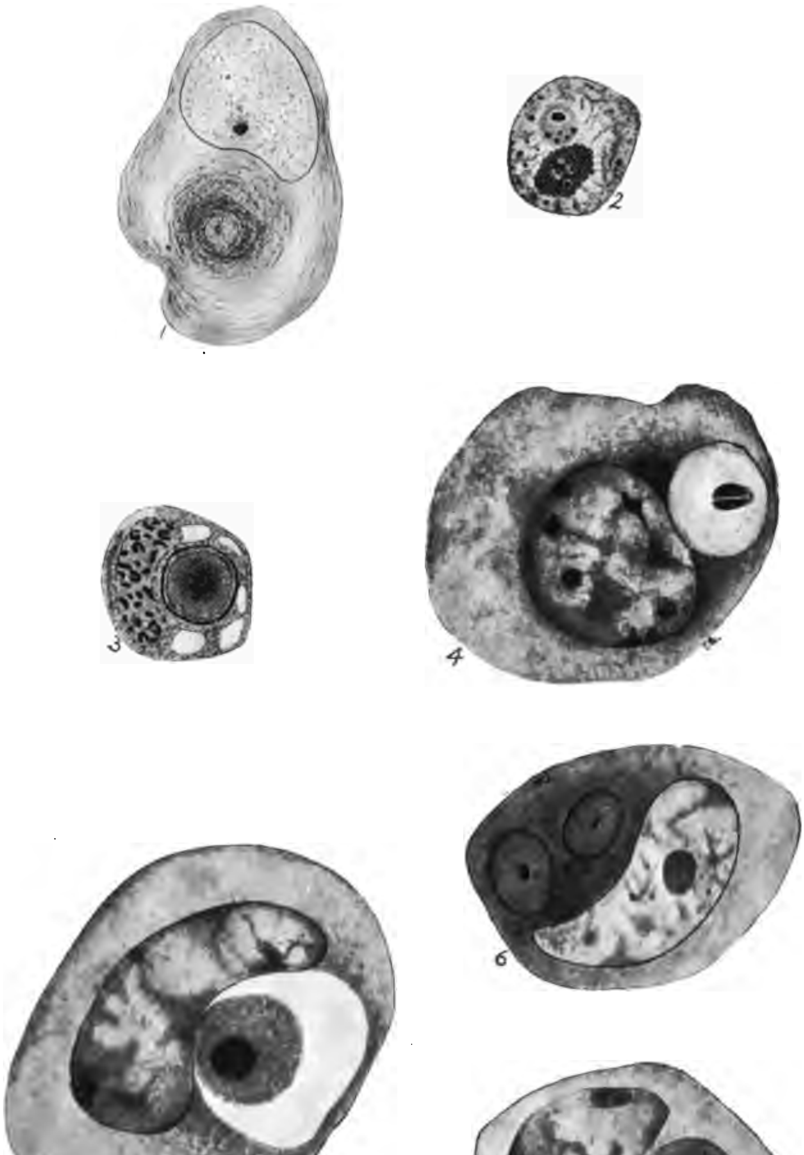
6. Plimmer's bodies: two in one cell body. Two are often met with, three occasionally, and more, rarely. Galeotti has studied multiple centrosome formation in karyokinesis of carcinoma cells.

7. Plimmer's body with an eccentrically placed nucleus. Notice the position of the body adjacent to the incurvation of the nuclear membrane, the usual position depicted in illustrations of the centrosome and attraction sphere.

Plate XXXVII.

8. Plimmer's body with a distinct capsule and large nucleus. The inside of the nucleus radiates like the astral rays or fibers of the archoplasm.

9. Showing a single large Plimmer's body, in the usual position in an incurvation of the nuclear membrane; the nucleus is very large — or the centrosome swollen — or the attraction sphere stains like the centrosome.



LECOUNT. CYTOPLASM.


8


9


10


11


12


13

(10–13. Copies of illustrations of centrosomes and archoplasmic structures of giant cells taken from Heidenhain, "Uber die Microcentren mehrkerniger Riesenzellen sowie über die Centralkörperfrage in Allgemeinen," Morph. Arb., 1897, vii, 225.) (The explanations of the figures are translations.)

10. Fig. 4, l. c., p. 233: Beautiful large giant-cell 35 by 27 μ dia.; centrally disposed hook-shaped microcentrum; nuclei in the periphery partially degenerated; to the left and above, large vacuoles, the remains of degenerated nuclei; the substance of the cell in granular degeneration. Dia. 2500.

11. Fig. 5, l. c., p. 234: Large giant-cell with multiple microcentra; the central bodies lying above and to the right appear to have arisen by division; the cellular destruction also begins in this region. Below, degenerated nucleus. Dia. 2500.

12. Fig. 8, l. c., p. 238: Small giant-cell with sphere and indication of Van Beneden's granule-layer; on the right border of the cell a semicircular impression made by a leucocyte; cell substance in granular degeneration. Dia. 2500.

13. Fig. 7, l. c., p. 237: Giant cell in complete degeneration. 36 by 22.5 μ dia.; length of the microcentrum, 10 μ. In the periphery a number of vacuoles containing nuclear remains; the substance of the cell in granular degeneration. Dia. 2500.

THE ENDOTHELIAL PHAGOCYTES OF THE TONSILLAR RING.

J. L. Goodale.

In the tonsillar ring two forms of phagocytic cells are encountered: first, polynuclear neutrophilic leucocytes, and second, large mononuclear cells, resembling morphologically epithelial cells. Both these forms of cells may be observed to incorporate foreign substances, with this difference, however, that whereas the leucocytes are phagocytic for bacteria, and for a variety of amorphus detritus, the epithelioid cells, on the other hand, are capable of incorporating living cells.

Attention was first called to the fact that certain cells may become phagocytic for other cells, by Mallory,[1] in a histological study of the lesions of typhoid fever in 1898. From his observations he concludes that dilute or weak toxines produce proliferation and phagocytosis in certain cells, particularly in the endothelial cells of lymphoid tissue, and of the lymphatics.

Several years ago, in a histological study of acute tonsilitis, I found in every case examined a more or less marked proliferation of the endothelial cells lining the reticulum of the follicles, with a formation of endothelial phagocytes. These findings led me to examine subsequently a large amount of material, with the object of ascertaining, first, whether these phenomena occurred also under normal conditions, and second, whether they bore any definite relation to infectious processes.

The following paper embodies the results of these observations.

For the satisfactory demonstration of the endothelial phagocytes and their inclusions, it is found best to place fresh tissue in Zenker's fluid, and to stain with alkaline methylene blue and eosin. Hematoxylin and eosin give much less perfect pictures.

Normal Histology. — In the study of the normal histology

tonsils were obtained at autopsy which presented, so far as could be determined macroscopically, no abnormality. Material was also obtained during life from cases requiring operation for adenoid hypertrophy, in which the tonsils exhibited clinically little or no enlargement. Finally a comparative study was made of the tonsils in some of the lower animals.

Man. — In the human subject, the tonsils show, in those places corresponding in situation to the follicles, a characteristic arrangement of the connective tissue cells. With Mallory's triple connective tissue stain, the framework of each follicle is in cross-section seen to be composed of a well-developed ring or capsule, composed of from four to six concentric layers of connective tissue. With ordinary acid stains as eosin, this fibrous envelope is difficult to isolate. It may be well demonstrated by neutral orcein. The follicles present, in sections of the tonsil made of the vertical to its base, a resemblance to a signet ring, with the seal directed towards the lumen of the nearest crypt. This appearance is due to the fact that the interior of the follicle is composed chiefly of endothelial cells, which owing to their large amount of cytoplasm are sharply differentiated from the encircling collection of lymphocytes. These endothelial cells undergo segmentation in a conspicuous manner, and produce large, so-called epithelioid cells. In every case which came under examination one or more of these phagocytic cells was found in the follicles. Where but one or two were present in the follicle, they were usually situated near the centre. As a rule, not more than eight or ten were found in a cross-section of an individual follicle. The incorporated substances existed almost exclusively of lymphoid cells and cellular detritus, probably derived from lymphoid cells; and were generally situated in clear spaces or vacuoles. Occasionally red blood corpuscles were found incorporated. Polynuclear leucocytes were not found in the cells, nor did the phagocytes appear to attack the endothelial cells. On the side of the follicle adjacent to the nearest crypt, the lymphocytes are especially numerous and closely packed. In this situation from four to six

rows of lymphocytes are found between each pair of concentric rings, which are thus more or less widely separated from each other. On the side of the follicle which faces towards the fibrous trunk, but one or two rows of lymphocytes are found between each pair of concentric rings. As one passes from the lymphoid cells of the capsule of the follicle, an alteration in these cells is noticed, consisting of a gradual increase in the amount of surrounding cytoplasm, which is usually more marked on one side, and appears to be heaped up more at the periphery than in the centre. The nucleus simultaneously undergoes alterations, consisting in a separation of its chromatin into deeply staining masses, which are situated in a rosette fashion, connected with each other by fine cromatin threads. These cells possess the characters of plasma cells. All stages of transition are observable, between the typical lymphoid cells and typical plasma cells. It was not, however, observed that these plasma cells were incorporated by the endothelial phagocytes. In the mucous membrane of the crypts numbers of plasma cells, lymphoid cells, and polynuclear neutrophiles were observed. In the fibrous trabeculæ near the base of the organ varying numbers of eosinophilic cells were usually encountered, but never in the neighborhood of the follicles or of the crypts.

Lymphoid tissue was also examined from other portions of the tonsillar ring. In the lingual tonsil, the lymph follicles are numerous, and the central reticulum of each follicle is well defined. An occasional endothelial phagocytic cell was found in the majority of specimens examined, but they were less numerous than in the faucial tonsils.

In examination of the pharyngeal tonsil from children, showing what may be called normal conditions, that is, occurring in the form of mammillated projections about one-eighth to one-quarter of an inch in height, it is seen that the central reticulum of the follicle shows but a slight tendency to proliferation, and the endothelial cells are sparingly encountered. In specimens obtained from adults at autopsy, from the vault of the pharynx, there is likewise observable a distinct central reticulum of the follicles, with an occasional endothelial phagocyte.

Comparative Histology. — Sections of tonsils from an adult opossum stained with polychrome methylene blue and eosin show the follicle to be indicated by a small group of cells, possessing a large amount of non-granular protoplasm, with large oval vesicular nuclei, surrounded by a ring of smaller cells, and possessing a narrow rim of protoplasm, with deeply staining nuclei. These cells correspond in appearance to the endothelial cells found in the human tonsil. Scattered among these cells are found considerable numbers of lymphoid cells, arranged in irregular lines together, with cells having the characters of plasma cells. In the germ center of each follicle are seen a few cells, resembling the phagocytes produced by proliferation in human tonsils. They consist of relatively large amount of acidophilic protoplasm, and with an eccentrically situated, lightly staining nucleus, and contain in their interior cells and cell fragments. The cells consist of lymphoid cells and plasma cells. The nuclei of the included cells appear compact and shrunken and stain deeply.

The follicle resembles closely that found in the human subject, differing chiefly in the smaller size of the germ center, and in the larger number of lymphoid cells found in the latter.

Sections of the follicles stained by Mallory's triple connective tissue stain show the germ center of the follicle to possess a well-developed fibrous reticulum, arranged in the form of irregular channels, containing these lymphoid cells previously referred to.

Examination of the tonsil from an adult cat shows but a slight differentiation of the follicles from the surrounding tissue. The germ centers are relatively large, and contain numerous large phagocytic cells, with a variety of inclusions, situated in the center of the vacuoles. The number of phagocytic cells in a cross section of the follicle ranges from ten to twenty. Red blood corpuscles are found frequently among the inclusions. Scattered among the endothelial cells are numerous lymphoid cells. Between the follicles are large numbers of large mononuclear phagocytes, crowded with

cellular inclusions and detritus. Some of the cells contain fifteen or twenty red blood corpuscles. The eosinophilic polynuclear cells, which occur in large numbers in the lymphoid channels, near the base of the tonsil, are at times incorporated by the phagocytic cells, but their granules disappear almost before they have been taken into the body of the phagocyte.

Staining by Mallory's method for connective tissue shows the development of the fibrous reticulum to be much less marked than in the case of the opossum, resembling in this respect more closely the human tonsil.

Several specimens of tonsil from yearling sheep were examined. The follicles resemble closely those found in the human tonsil, possessing a distinct endothelial reticulum, with surrounding lymphoid cells. Endothelial proliferation and phagocytes were present in the majority of follicles, particularly near their center. The inclusions were chiefly lymphoid cells and cellular detritus, although at times polynuclear leucocytes appeared to be incorporated. The fibrous tissue is well developed in the follicles.

Several specimens were examined from cattle. The tonsils here exhibit a penetration of mucous glands in all directions in the lymphoid tissue, but the individual follicles exhibit the ordinary type. The fibrous reticulum of the follicles is poorly developed, and proliferation varies in amount in different follicles. In some the mitotic figures are numerous, while in others none are found in cross section. The phagocytes resemble essentially those of the sheep, and contain inclusions of lymphoid cells and cellular detritus.

PATHOLOGICAL HISTOLOGY.

Hyperplasia.

Of the pathological conditions of the tonsils it seems desirable to describe first simple hyperplasia, since there is no line which separates it sharply from the normal tonsil, and especially since many of the pathologically altered tonsils which are shortly to be described were to a greater or less extent hyperplastic.

We have to consider two forms of hyperplasia: first, diffuse hyperplasia, and secondly, the so-called papillary hypertrophy.

In simple hyperplasia the most conspicuous alteration in the tonsil is a great increase in the number of the endothelial cells of the reticulum. There is also an increase to a less extent of the interfollicular endothelial cells. The lymphoid ring is, however, not increased proportionately. It even gives, at times, the appearance of a thinning, as if due to an enlargement of the central reticulum. In the reticulum, as shown by saffranin, proliferation of the endothelial cells is extremely marked, and is in proportion to the degree of hyperplasia present. The endothelial phagocytes are most numerous near the center of the reticulum, and are crowded with lymphoid cells and cellular detritus to a varying degree. Where in addition to the hyperplasia there is an associated cheesy collection in the tonsillar crypts, the endothelial phagocytes appear to be correspondingly increased in number, thirty or forty having been observed in a cross-section of a single follicle. In such cases the number of polynuclear leucocytes in the crypts is increased, and are found to be incorporating bacteria to a greater or less degree. Staining of the tissues by Gram shows, however, no penetration of the bacteria below the epithelium, and in no cases were bacteria found in the endothelial phagocytes. The lining endothelium of the capillaries and blood vessels showed no unusual proliferation.

Two cases of papillary hypertrophy were examined. In the first case a tonsil enlarged to the size of a pigeon's egg, and irregularly lobulated and mammillated, presented on histological examination a marked proliferation of the mucous and sub-mucous tissue, the former being prolonged upward into delicate papillæ. The endothelial lining of the connective tissue showed active proliferation. Not only is the mucosa in a state of active proliferation, but the submucosa as well, which throws upward long digitations above the surrounding level of the mucous membrane. The follicles are much enlarged, owing to a proliferation of their endothelial

reticulum, and phagocytic cells are abundant. The lymphatic ring surrounding each follicle shows no especial widening, and there is no unusual collection of lymphoid or plasma cells in the interfollicular spaces, although the endothelial cells are here in a state of heightened proliferation. The crypts are filled with detritus, leucocytes, and bacteria. Sections stained by Mallory's method for connective tissue show the papillæ of the mucosa to be largely composed of connective tissue fibers, lined with endothelial cells. There is no abnormal development of connective tissue in the center of the follicles, the stain showing the enlargement of the latter to be due essentially to a heightened proliferation of the endothelial cells. The larger fibrous trunks of the tonsil show a heightened activity of their fibroblasts, and are in direct communication with the digital prolongations previously described.

A case of papillary hypertrophy previously described by Dr. J. P. Clark shows on microscopical examination a marked proliferation of the connective tissue of the mucosa as well as of the lining endothelial cells. The epithelial layer shows numerous mitoses, and here and there isolated degeneration of the cells. It sends down interpapillary prolongations which contain very numerous mitotic figures. The mucosa proper is crowded with lymphoid cells, as are also the lymphatic channels in the vicinity, many of which are widely dilated with these cells. The mucous membrane contains many polynuclear neutrophiles, fixed in the interspaces. The submucosa shows a marked development of blood vessels which are distended with red blood corpuscles, lymphoid cells, and polynuclear neutrophiles.

The condition resembles closely that found in the preceding case, both in the development of the endothelial and connective cells, but does not apparently reach so marked a development. It differs from it most noticeably in the much greater infiltration of the lymph spaces with lymphoid cells.

Retrograde Metamorphosis.

A series of tonsils from adults were examined, with regard to the phenomena exhibited by the endothelial cells of the reticulum. These cases have been reported in detail,[2] and only a brief résumé of the points in question will be given here. In atrophied tonsils, the follicles are markedly diminished in number. Those which are situated near the crypts are the largest, and show most active proliferation of the endothelium. Those follicles, on the other hand, more remote from the crypts are smaller, and show little or no tendency of the endothelial cells to proliferate. The follicles which are near crypts provided with a loose, mesh-like epithelium are more active than those follicles in the vicinity of the crypts lined with a more compact epithelium. In the interior of the tonsil the fibrous changes are usually more marked than near the periphery, and traces may be seen here of follicles, the germ center of which has disappeared, with the exception of a few endothelial cells, surrounded by a small collection of lymphoid cells.

Acute Inflammation.

Inflammatory processes affecting the faucial tonsils were studied under two forms: first, those occurring primarily through infection, and secondly, those produced by trauma.

In acute infectious tonsilitis the endothelial center of the follicle appears enlarged from an increased proliferation in the endothelial cells. The number of phagocytes in each follicle ranges (in a section 2 μ in thickness) from ten to fifty or more. The inclusions in these cells do not apparently differ from those found in normal and in hyperplastic tonsils, consisting chiefly of lymphoid cells, cell fragments, and fine granular detritus. At times plasma cells appear to be incorporated. It must, however, be remembered that many of the tonsils were originally hyperplastic, and must have presented, therefore, an increased endothelial proliferation, before the onset of the acute inflammation. The appearance, in fact, of the endothelial reticulum of the follicle

does not differ materially from that found in many cases of hyperplasia. A marked point of distinction was, however, present in all cases, and consisted in a proliferation of the endothelial cells of the capillaries and small vessels. These endothelial cells exhibited frequently swelling and desquamation. They were at times found lying free in the lumen of the vessels. Another point of distinction observed in these cases was the widening of the ring of lymphoid cells around each follicle. These lymphoid cells occurred also in greater numbers in the lymph channels between the follicles.

In the less frequent cases of suppurative tonsilitis, which are characterized by the presence of multiple abscesses within the follicles, examination shows the following conditions: The first indication of an abscess in a follicle consists in circumscribed infiltration of polynuclear leucocytes among the endothelial cells of the reticulum. The blood vessels in the immediate neighborhood contain large numbers of polynuclear leucocytes, some of which are seen in the act of passing through the vessel wall. The endothelial cells of the vessel show a varying amount of proliferation in swelling. Micrococci are found in varying numbers in the region occupied by the polynuclear leucocytes, lying for the most part free in the intercellular spaces, although they not infrequently may be seen in the interior of the polynuclear leucocytes, and apparently also within the large endothelial phagocytes. With the growth of the abscess the follicle increases in size, as the result both of heightened emigration of polynuclear leucocytes and of the proliferation of the surrounding endothelial cells of the reticulum. The endothelial cells of the reticulum in the immediate neighborhood of the abscess show a swelling of their cytoplasm, and an irregularity in the outline of their nucleus, which appears elongated, indented, or twisted. A marked increase is simultaneously observed in the number of endothelial phagocytes in the vicinity, which contain also a greater number of incorporated lymphoid cells and cell fragments.

Investigations were made on human tonsils to determine the effect of irritation upon the proliferation of the endothelial

cells of the reticulum. In a series of six cases, a drop of chromic acid was introduced into the crypt of a tonsil, which exhibited sufficient hypertrophy to require excision, and at a varying period of time afterwards the tonsil was removed and examined histologically. In all the cases examined, a necrosis of the epithelium of the cauterized crypt was observed, extending for a varying distance into the underlying lymphoid tissue. In the center of the necrotic area there were no polynuclear leucocytes, but towards the periphery these were encountered in large numbers. Bacteria were numerous throughout the necrosed area, and in the region of the infiltration many polynuclear leucocytes were seen, usually with bacteria in their interior. Below the area of necrosis a number of lymphoid and plasma cells were observed, together with many polynuclear neutrophiles, the latter emigrating from the dilated blood vessels of the vicinity. The endothelial cells of the reticulum showed nuclear degeneration and fragmentation, but no evidence of phagocytosis was observed. The follicles in this vicinity presented traces of their endothelial cells in the form of acidophilic rings, corresponding to the preëxisting cell walls, filled with amorphus homogeneous material. Below this region, at a greater distance from the site of the cauterization, the follicles show an increased endothelial proliferation of the reticulum and of the intima of the blood vessels, with a formation of many phagocytes.

The number of cases observed was too small to justify conclusions regarding the relative intensity of these phenomena in the different stages of the process.

Conclusions.

From these observations it is evident that in normal tonsils, both of man and of a variety of the lower animals, large mononuclear phagocytes are present, which appear to be derived from a proliferation of the endothelial cells of the reticulum.

In hyperplasia of the tonsils there is a proportionate increase in the proliferation of the endothelial cells of the retic-

ulum, and formation of phagocytes, without, however, a corresponding increase in the number of lymphoid cells, or in the endothelial cells of the blood vessels.

In atrophy of the tonsils, the endothelial cells of the follicles are seen first to diminish in number, and endothelial phagocytes are correspondingly few, while the lymphoid cells persist relatively longer.

In acute inflammation, the characteristic feature of the process consists in a heightened proliferation of the endothelial cells of the blood vessels, with swelling of their cytoplasm, together with an increased number of lymphoid cells. These changes are relatively more marked than the increased endothelial proliferation of the reticulum with follicles. Where circumscribed abscess formation occurs in the follicles, an increased proliferation of the endothelial cells, both of the reticulum and the capillaries, is observable in the vicinity.

Where an intense irritant acts upon the endothelial cells of the tonsil, degeneration and necrosis result in the immediate vicinity. At a greater distance proliferation appears to be excited in these cells.

It seems, therefore, probable that proliferation of the endothelial cells of the reticulum, with a formation of phagocytes, is not necessarily dependent upon the influence of bacterial toxines for its production, but appears rather to stand in a definite relation to the size or activity of the organs in question. Proliferation, on the other hand, of the endothelial cells of the blood vessels was found to occur only in association with other phenomena of inflammation.

(My thanks are due to Dr. C. S. Minot for facilities kindly afforded me in his laboratory in the Harvard Medical School.)

REFERENCES.

1. Mallory. Journal of Experimental Medicine, vol. iii, No. 6.
2. Goodale. Archiv. für Laryngologie, Band xii, Hft. 3.

ON THE ACTIVE PRINCIPLE OF JAMAICA DOGWOOD.

M. Vejux-Tyrode.

(*From the Pharmacological Laboratory of the Harvard Medical School and Chemical Laboratory of the Massachusetts General Hospital.*)

In several wild countries the natives use various poisonous plants to catch fish for food purposes. They make extracts of these plants which they throw into the streams. The fish becomes narcotized or killed and floats to the surface of the water, when it may be picked up with nets. Among these plants is anarmita paniculata, the mother substance of picrotoxin. Another one is timbo, which is used by the Indians along the banks of the Amazon. Still another of these plants is piscidia erythrina or Jamaica dogwood, which is used as a fish poison by the natives of some of the West Indies.

In 1883 a chemist, E. Hart, published an article in which he claimed to have found the active principle of Jamaica dogwood in the form of a white crystalline body which he called piscidin. His work was, however, not beyond criticism.

In view of the fact that practically nothing was known about the active principle of Jamaica dogwood, two years and a half ago, Professor Pfaff suggested that I should investigate this plant, which I did. While working on this research there appeared in the "American Chemical Journal," 1901, a publication of a very commendable chemical investigation of Jamaica dogwood by P. C. Freer and A. M. Clover. Their method of investigation was quite different from the one which I pursued. They isolated several bodies, some of which were decomposition products and derivatives which they formed. Amongst others they investigated two crystalline bodies, a mixture of which they believed formed the piscidin of Hart. They submitted their products for pharmacological experiments to Professor Cushny, who failed to find any activity in them.

I made various extracts of Jamaica dogwood with dilute

alcohol, ether, and chloroform, and found a number of bodies among which was a white crystalline body of an average percentage composition of C 67.26%, H 5.19% almost insoluble in water and petroleum ether, and ether, sparingly soluble in alcohol, very soluble in chloroform.

When first tested this body seemed to show activity towards frogs, but after further purification ceased to show any more action. Of all the bodies which I investigated so far the only one which possesses undoubted pharmacological action is a light brown resin which when sufficiently purified can be reduced to a light yellowish brown powder by rubbing with petroleum ether. On standing or after heating on water bath this body gives rise to a light yellow body which is inactive towards frogs in the dose of 0.006 to 0.010 grammes.

In the main the results of the pharmacological investigations of the resin are the following:

After a dose of from 0.002 to 0.004 gramme of the purified resin containing no ash, a frog becomes narcotized in from one to two hours. Stupor and paralysis gradually increase until respiration stops. The heart still beats for some time after the animal is apparently dead.

When fishes are placed in water containing in suspension one part of the resin to twenty-five thousand parts of water, gradual narcosis comes on with death in a few hours.

Medium size rabbits show no symptoms after either the subcutaneous or the internal administration of from 0.05 to 0.3 gramme.

A dog of about eight kilos body weight showed no definite symptoms after the administration of 0.5 gramme subcutaneously.

Although no symptoms could be obtained in the doses given to warm-blooded animals, still there can be no doubt of the great activity of this substance towards the cold-blooded animals, frogs, and especially fish.

This disproportionately greater toxicity towards cold-blooded animals makes quite a striking feature in the action of this substance.

I am now more closely investigating the chemical characteristics of this highly interesting resin and hope soon to follow this merely preliminary communication by a more detailed description of the chemical and pharmacological properties of this substance.

THE LYMPHOMATOUS TUMORS OF THE DOG'S SPLEEN.[1]

HERBERT U. WILLIAMS, M.D., AND FREDERICK C. BUSCH, M.D.

(*Medical Department, University of Buffalo.*)

Although primary tumors of the spleen are rare in man, this is not the case in the dog. The observations of the writers are based on autopsies on seven hundred and twenty apparently healthy dogs. In seventeen of these, or two and one-third per cent, tumor-like nodules were present in the spleen. None of the dogs gave macroscopic evidences of leukemia or pseudo-leukemia, or showed enlargement of lymphatic glands or other lymphoid structures; nor were lymphoid deposits visible in other organs.

The number of tumors seen in a single spleen varied from one to seven. In one case moderate enlargement of most of the Malpighian bodies co-existed with the splenic tumors in question. Two or three enlarged Malpighian bodies were a few times detected in spleens containing the tumors, but this was not the rule. The tumors usually occurred in otherwise normal spleens.

In shape they were roughly spherical, in diameter from five millimeters to four centimeters. On section they proved to be composed of small, soft, white, translucent masses, resembling Malpighian bodies, but larger. They contained also a variable amount of a soft, dark red substance, looking like spleen-pulp or blood-clot, in which the translucent masses were embedded. The latter were found to consist of lymphoid tissue. The soft reddish substance appeared to correspond with the splenic pulp; in some tumors the amount of it was small; in its meshes were red blood-corpuscles and sometimes considerable masses of blood; it contained a very variable quantity of pigment.

With low magnification the structure of the tumors was

[1] Read March 29, 1902, at the Second Annual Meeting of the American Association of Pathologists and Bacteriologists, at Cleveland, Ohio.

seen to differ from that of the spleen in three important particulars:

1. In the absence of trabeculæ except at the edges.

2. In the large size of the lymphoid masses as compared with the Malpighian bodies of the normal spleen.

3. In the absence of a central artery in the lymphoid masses as a rule, though not invariably.

It is noteworthy that areas of necrosis or caseation were not encountered in the tumors.

In six cases microscopic examinations were made of the liver, kidney, and lung for deposits of lymphoid tissue. Nothing of the kind was found with the exception of a few small collections of lymphoid cells between the tubules of the kidney in two cases.

In four cases smears were made of the heart's blood; the results were not important.

In six cases bacteria were sought for in the tumors in stained sections and by cultures, with negative results.

Although a number of attempts were made to inoculate portions of the tumors into the spleens of other dogs, the circumstances were satisfactory in one case only. The results of this experiment were negative, the spleen showing nothing but small scars at the end of eleven and one-half weeks.[1]

While collecting our material we encountered a number of cases of hemorrhages in the dog's spleen (hemorrhagia intrapulposa subcapsularis, Kitt), which superficially resembled the tumors above described. Sometimes it was even difficult to decide whether we were dealing with a blood-clot in process of resolution and being invaded by granulation tissue, or with a new growth of atypical spleen-pulp and lymphoid tissue. One such case, which we classified as hemorrhage, showed undoubted multiplication of the lymphoid elements remaining in the clot. The absence of trabeculæ from the genuine tumors and the presence of blood in them will be recalled. These facts suggested the possibility of a traumatic

[1] Incidentally to this work we secured an atypical adenoma of a dog's breast, pieces of which were introduced into a normal dog's breast while very fresh and having been kept warm. The wounds made healed quickly, but no growth resulted after seven weeks.

origin for some of the tumors, although the inherent improbability of this theory was appreciated. The absence of conspicuous pigmentation from the tumors was also an argument against this theory.

There seemed, however, sufficient encouragement to warrant our observing the progress of experimental lacerations. The results of the experiments in this direction (ten in all) were negative. When the spleen was well lacerated beneath the capsule and the capsule was torn away from the pulp, quite marked blood-tumors formed in a few minutes; no trace of them was visible after two or three weeks except a pigmented scar. The result was similar in two cases where the veins leaving the lacerated portion were tied.

The growths described above are not mentioned in many recent text-books on pathology. They are alluded to briefly in the well-known works of Orth and Birch-Hirschfeld. They are more fully described by Kitt.[1]

According to Kitt, the lymphoid tumors of the spleen (excluding those which occur in leukemia and pseudoleukemia) may be of two sorts:

1. A universal hypertrophy of the Malpighian bodies, with much enlargement of the spleen (hyperplasia follicularis splenis, splenoma, splenadenoma, etc.).

2. Circumscribed tumors of lymphoid tissue, which may be single or multiple (lymphoma). The names "malignant lymphoma" or "lymphosarcoma" are also used for the second variety, apparently when the tumors are multiple and produce great enlargement of the spleen. Both kinds are common in the dog, hog, cow, and horse. The tumors we have encountered seem, with one exception, to correspond with the second class mentioned, though their clinical tendencies were certainly not malignant. Apparently there are many links by which the two varieties are connected.

(We are indebted to Dr. A. T. Kerr, Assistant Professor of Anatomy, Cornell University, Ithaca, for furnishing us much material.)

---

[1] Lehrbuch der pathologisch-anatomischen Diagnostik für Thierärzte, Bd. ii., and Pathologische Anatomie der Hausthiere, 2te aufl, Bd. ii., pp. 398, 404.

UPON AN EXTENSIVE OUTBREAK OF FOOD INTOXICATION AND INFECTION OF UNIQUE ORIGIN.[1, 2]

A. P. OHLMACHER, M.D.

(*Professor of Pathology, Northwestern University, Medical School, Chicago, Ill.*)

Introduction and Statistics.

During the three days succeeding November 29, 1897, one hundred and eighteen male and one hundred female inmates of the Ohio Hospital for Epileptics at Gallipolis became acutely ill and took to bed. A considerable number of other inmates were also affected by the prevalent sickness, but were not confined by it.

A few words as to the plan of the hospital will here be in place. The institution was built on the so-called "cottage plan," with two wings composed, at that time, of six cottages for male and six for female patients, accommodating about fifty patients in each cottage. Cottage Six, on the female side, and Cottage G, on the male side, were "closed cottages," provided with independent kitchens and dining-rooms. With the exception of a few better class patients in Ward I of Cottage Six, all the inmates of the closed cottages ate in their respective separate dining-rooms. For the inmates of the open cottages two central congregate or general dining-rooms (with one common kitchen) were situated in two buildings (Dining-Room Buildings), located midway between the two groups of cottages. One of these general dining-rooms was for males, the other for females. Adjacent to each dining-room was a serving-room with a steam warming-table. The serving-room adjoining the dining-room for females was fifty feet wide and forty feet long. The ceiling was about twenty feet high, of lath and plaster, with a calcimined surface, and was divided into two equal portions by a transverse

[1] Read, by title, March 29, 1902, at the Second Annual Meeting of the American Association of Pathologists and Bacteriologists, Cleveland, Ohio.

[2] These observations were pursued in the Pathological Laboratory of the Ohio Hospital for Epileptics.

beam or girder. Three sets of double doors opened from this serving-room, two into the general dining-room, and one on to the main hospital thoroughfare which at this time was an unpaved dirt road. A large steam- or warming-table, ten feet long by thirty-two inches wide, was located in the center of this serving-room. In the steam-table were seven pans, the four larger ones being twenty-six inches long, eighteen inches wide, and ten inches deep. Each pan was covered with a loosely-fitting tin lid. No provision for the escape of steam from the warming-table had been made, and during its operation dense clouds of steam filled the serving-room and condensed on the ceiling and walls.

The resident patient population and the sick rate before and after November 29, 1897, is shown in the subjoined tables:

TABLES SHOWING PATIENT POPULATION AND SICK RATE BEFORE AND AFTER NOVEMBER 29, 1897.

TABLE I.

From the Census Book for 1897:

Nov. 27.	Total male population	335
	" female population	272
	Grand total	607

TABLE II.

From the Supervisor's books:

Male Department.

November 27, 28, 29, 1897, patients in Cottage G[1] . 82

Sick in bed on the following dates (not including paralytics and chronic bed-ridden):

Nov. 27,	*2*	Nov. 28,	*7*	Nov. 29,	*6*	Nov. 30,	*89*
Dec. 1,	*111*	Dec. 2,	*118*	Dec. 3,	*112*	Dec. 4,	*58*
Dec. 5,	*48*	Dec. 6,	*21*	Dec. 7,	*18*	Dec. 8,	*12*
Dec. 9,	*20*	Dec. 10,	*17*	Dec. 11,	*15*	Dec. 12,	*17*
Dec. 13,	*15*	Dec. 14,	*12*	Dec. 15,	*13*	Dec. 16,	*11*
Dec. 17,	*5*	Dec. 18,	*10*	Dec. 19,	*7*	Dec. 20,	*8*
				Dec. 24,	*4*		

[1] Cottage G was the closed cottage for males.

Average male capita sick rate from acute illness about this period was five.

On November 29, two hundred and fifty-one men ate in the general dining-room.

TABLE III.

From Supervisor's books:

Female Department.

November 27–29, patients in Cottage Six[1] 82

Sick in bed on the following dates (acute cases):

Nov. 27,	*3*	Nov. 28,	*3*	Nov. 29,	*4*	Nov. 30,	*31*
Dec. 1,	*80*	Dec. 2,	*100*	Dec. 3,	*55*	Dec. 4,	*51*
Dec. 5,	*23*	Dec. 6,	*19*	Dec. 7,	*21*	Dec. 8,	*20*
Dec. 9,	*16*	Dec. 10,	*14*	Dec. 11,	*10*	Dec. 12,	*12*
Dec. 13,	*10*	Dec. 14,	*12*	Dec. 15,	*12*	Dec. 16,	*9*
Dec. 17,	*9*	Dec. 18,	*8*	Dec. 19,	*9*	Dec. 20,	*7*
Dec. 21,	*7*	Dec. 22,	*7*	Dec. 23,	*7*	Dec. 24,	*7*
Dec. 25,	*5*	Dec. 26,	*2*	Dec. 27,	*3*	Dec. 28,	*1*

Average capita sick rate on female side for month October 29–November 29, 1897, before food poisoning, five.

On November 29, two hundred and fifty-five women ate in the general dining-room.

Clinical Manifestations.[2]

Beginning the afternoon of November 29 (Sunday), and becoming fully pronounced during the next twelve hours, the following symptoms were noted: Chilliness, especially up and down the spine, cold hands and feet. Aching of limbs. Severe headache and sense of pressure in the head. Nausea, and vomiting in many cases, but not in all. Pain in abdomen, especially about umbilical region, griping and cramps. Profuse watery diarrhœa. Soreness of abdomen. Dizziness and staggering gait. Prostration. Fever.

[1] Cottage Six was the closed cottage for females, with Wards 1 and 2.

[2] For clinical data I am under special obligation to Drs Richard O'Connell, W. G. List, and S. J. Webster, assistant physicians. A complete temperature record in 118 male patients with two daily readings for the week following the outbreak of the sickness was furnished by Dr. O'Connell.

The tongue showed a slight whitish coating. The pulse varied from 100 to 120 beats a minute. The respiration was accelerated in keeping with the fever. The average rise of temperature was to 102.5° F., the maximum 105° F., minimum 100° F. In all cases the fever persisted four days, in some two weeks elapsed before the normal temperature was reached. The stools were fluid, at first yellow and smooth, then choppy, and not particularly fetid. In some cases the diarrhœa persisted for two weeks. The vomitus at first consisted of ingested food, then it became more fluid, yellowish, and finally greenish. A number of patients were severely prostrated. None died.

In the mild cases, of which an exact record was not kept, the symptoms like nausea and looseness of the bowels were transient, and these patients were not compelled to take to bed, and were not included in the above-shown tables. Some patients among those eating in the general dining-rooms escaped entirely.

Examination of Food Articles Originally Suspected.

Within a few hours after the onset of the illness among the patients it was evident that we were dealing with a case of wholesale poisoning, presumably through some article of diet. Before the symptoms (and especially the fever) became sufficiently pronounced it was thought that some mineral poison had been ingested, though the later developments pointed to an intoxication by bacterial products or ptomains and an infection with viable pathogenic bacteria. As soon as food-poisoning was settled a laboratory investigation was instituted, the preliminary work beginning during the evening of November twenty-ninth.

Three articles of diet fell under suspicion on the first impulse, viz.: the milk, the butter, and the apple-butter. The butter and apple-butter were first tested for arsenic and copper, with negative results. A specimen of vomitus was similarly examined without positive result. Then, during the three days succeeding November twenty-ninth, the milk and butter from two lots were examined by evaporating ethereal

extracts after the method employed by Vaughan[1] for tyrotoxicon in milk. The viscid, milky residue thus obtained was administered by the mouth to cats and rabbits in doses varying from two to five cubic centimeters, but no toxic effects were observed.

The Discovery of the Probable Real Cause.

It was after a careful inquiry into the existing conditions, and by a process of exclusion, that the probable factor in this outbreak of food-poisoning was discovered, and as the conclusion was not reached until December third, four days after the sickness appeared, none of the particular lot of food under suspicion and none of the vomitus from the patients could be secured for examination. But experimental testimony of confirmative value was obtained in a roundabout manner.

As soon as all the cases of illness had developed it became evident, first, that only the patients in the institution were affected; and, second, that of the patients, only those eating in the central general dining-rooms (including several from Ward I of the female closed cottage) suffered, while the officers, employees, and the patients in the closed cottages (in other words, those eating in the various separate dining-rooms) escaped. This circumstance made it possible to definitely exclude most of the articles figuring on the bill of fare at this period, for these articles like milk, butter, meats, vegetables, and canned goods were used in all the separate kitchens in the institution, and were issued from a common stock, so that had milk or meat, for instance, been at fault, the sickness should have appeared amongst all the patients, the officers, and the employees. For the same reason no suspicion was attached to the drinking water, which was common to the whole hospital, and from a single source.

In looking over the bill of fare for November twenty-eighth and twenty-ninth it was found that the only article peculiar to the central or general dining-rooms was the oatmeal, which, with bread and butter, apple-butter, and coffee, constituted

[1] Vaughan and Novy. Ptomains, Leucomains, Toxins and Antitoxins. 1896, p. 99.

the breakfast for November twenty-ninth. It was further learned that while the oatmeal, a regular morning article of diet, was issued from a common stock to all parts of the hospital, it was separately cooked for the officers, employees, and in each of the kitchens of the closed cottages. At this period, however, all the oatmeal used in the general dining-rooms for male and female patients was cooked in the serving-room adjoining the dining-room for females, the arrangements of which have already been described. Thus it was decided that the batch of oatmeal used in the central dining-rooms for breakfast November twenty-ninth was, in all probability, the offending article of food. What were the conditions which rendered this particular batch of oatmeal dangerous?

The Contamination of the Oatmeal.

At this period it was customary to prepare the oatmeal for the general dining-rooms by steaming the batch of twenty gallons in three of the large pans on the steam-table during the entire afternoon. About six o'clock P.M. the steam was turned off and the oatmeal gradually cooled, reaching the body heat about nine or ten o'clock P.M. In the morning it was warmed by a brief steaming before breakfast. Now the lot of oatmeal used on November twenty-ninth was exposed to entirely unusual conditions.

As has already been related, there was no adequate means of egress for the steam arising from the steam-table, and as a consequence the plaster on the ceiling of the serving-room gradually became loosened. It finally became necessary to repair the ceiling, and this was undertaken at ten o'clock Saturday evening, November twenty-eighth, when six hundred square feet of old plaster was knocked off and replaced with new. The removal of this old plaster, which fell to the floor from the twenty-foot ceiling, was naturally attended by the production of an enormous amount of dust. While this operation was in progress, the oatmeal for the next breakfast was in the steam-table, where it had cooled to about the body temperature. It was contained in the three large pans, covered with their loosely-fitting tin lids, and, as a further

protection against the plaster, some sheets of paper and some rubber sheets were laid over the exposed surface of the steam-table. This covering sufficed to prevent the entrance of larger masses of plaster, but did not prevent the admission of the fine plaster dust which sifted in, and insinuated itself everywhere throughout the room. Unquestionably this batch of oatmeal was contaminated with dust from the ceiling, and its temperature at this time was about that of the incubator, which probably was maintained longer than usual by virtue of the additional covering over the steam-table. But even granting all this, how could the plaster dust affect the oatmeal so as to render it poisonous?

It has already been stated that the ceiling of this serving-room was exposed to peculiar conditions, for, owing to the inadequate provisions for the escape of steam from this room, the ceiling was daily moistened and warmed by the clouds of vapor arising from the cooking. The summer of 1897 had been unusually dry and dusty, and the proximity of the serving-room to the main hospital thoroughfare was such as to expose it directly to the clouds of dust arising from the unpaved road and entering the open doors and windows. This dust readily found lodgment on the moist ceiling, and one may also conceive that the bacterial inhabitants of this dust found excellent vegetative conditions in the daily moistening and warming by the steam and the vapors of cooking. At least such was the upshot of our reasoning, as the various conditions above related were disclosed and analyzed, and at this stage the effort to obtain experimental proof was made.

Bacteriological Examination of the Plaster Dust.

Experiments, Series I. — Only half of the ceiling had been replaced. Accordingly, on December third and fourth, pieces of the old plaster still remaining were obtained with bacteriological precautions and powdered in a sterilized mortar. At the same time scrapings of dust were obtained from the old calcimined surface of the ceiling. With this material a series of flasks and tubes of bouillon were inoculated; some were placed in the incubator, others not. Those kept at the

room temperature developed a moderate bacterial growth in twenty-four hours, while those in the incubator became extensively clouded and, without exception, produced a foul putrefactive odor. From this incubated bouillon plate cultures were prepared in two sets, one from the bouillon inoculated with the powdered plaster, the other with the scrapings from the surface of the ceiling. In each of the two sets of plates two bacterial species predominated which on isolation and subsequent identification proved to be Bacillus coli communis, and a rapidly liquefying, non-chromogenic, putrefactive, gas-producing, motile bacillus of the *Proteus* group, most closely resembling Proteus vulgaris.

The pathogenicity of the microbes developing in the incubated bouillon contaminated with the scrapings was tested by intra-peritoneal injection of one-half to one cubic centimeter of the twenty-four-hour mixed culture into three guinea-pigs, producing septic peritonitis and death in each case in twenty-four to thirty-six hours.

Besides this indirect mode of isolating the bacteria of the plaster dust, B. coli and Proteus vulgaris were recovered in plate cultures prepared by shaking the plaster dust in bouillon and making dilutions of such a suspension.

It was, therefore, clear that the plaster dust suspected as one of the chief harmful factors in this example of food-poisoning harbored at least two pathogenic bacterial species. Could these same bacterial species, mixed with the other microörganisms present in the plaster dust, so contaminate a batch of oatmeal as to make it dangerous as an article of food?

Experimental Contamination of Oatmeal.

Experiments, Series II. — Two lots of oatmeal, about two quarts in each, cooked by the usual procedure as for consumption, were placed in suitable sterile glass dishes. Into the oatmeal of Lot A plaster dust obtained by scraping the ceiling was sifted. Lot B was not so treated. Both were kept in the incubator twelve hours. Small portions from each lot of oatmeal were then fed directly to rabbits and cats

with no appreciable effect. Both lots were now subjected to extraction by the Stas-Otto method for obtaining ptomains. The extract from Lot A was a yellowish oily fluid, that from Lot B was more fluid and milky. The extract from Lot B was administered to three cats in quantity from a few drops to several cubic centimeters. No harmful effect was noted.

In each of three cats a few drops of the extract from Lot B were placed on the tongue. Each of the animals had a rise of temperature of two or three degrees Farenheit within half an hour. One cat vomited in fifteen minutes. In another a diarrhea, persisting for several days, was set up. In the third, fibrillary tremor, rapid breathing, convulsions, and opisthotonos followed in half an hour after the extract was administered.

General Remarks.

From the clinical standpoint it seems quite clear that the premonitory symptoms were those of an intoxication, while the later ones and particularly the fever which persisted should be ascribed to infection with living pathogenic bacteria. It is not certain in how far the colon bacillus or the bacillus of the Proteus group are to be held accountable, but the evidence at hand seems to indicate that one or the other of these species or both in combination, perhaps together with some other undetermined microörganisms, were the chief exciting agents. As for the Proteus group, it has already been looked upon as occasionally producing food-poisoning, particularly certain cases of boutilismus.

It will be observed that somewhat less than half the individuals reporting for breakfast on the morning in question became sick enough to take to bed, and that, all told, not more than sixty per cent of those eating this meal took ill. This apparent contradiction to the accepted explanation of the origin of the outbreak may be eliminated on the basis that only the individuals seriously affected ate of the oatmeal, or at least partook of it in sufficient quantity to produce disturbance. Oatmeal was a standard article of morning diet in

the institution, and a good many patients did not eat it either from prejudices of taste or on account of its monotony. The male inmates suffered more seriously both in point of numbers and in severity of symptoms. This is to be explained by the fact that they were larger eaters, many, indeed, being gluttons, without the more fastidious tastes and smaller appetites of the women.

No exact parallel to the case here recorded can be found, and this is scarcely surprising in view of the extraordinary conditions leading up to the accident. Several instances are cited (and noted in Vaughan and Novy's Ptomains and Leucomains) in which certain kinds of meal, or bread made from such meal, produced illness, but in these cases the meal had become musty or otherwise damaged in the raw state.

Recapitulation.

In the above-described case two hundred and eighteen patients in a public institution became acutely ill with symptoms of food-intoxication and of gastro-intestinal infection. By a process of exclusion or elimination the offending article of food was finally decided to be a certain batch of oatmeal. It was found that this particular batch of oatmeal had been contaminated by the dust arising from the removal of a large section of plaster from a ceiling. This ceiling had been exposed to clouds of dust from a dirt road, and had been constantly exposed to steam and the vapors of cooking. The surface of this ceiling and the dust of its plaster harbored living bacteria, among them B. coli communis and Proteus vulgaris. It is to the presence of these bacteria and their multiplication in the oatmeal (where they were carried by the plaster dust) that the toxic and infectious character of this article of diet is ascribed. Experimental evidence in favor of this theory is adduced.

THE HISTOLOGIC AND HISTOGENETIC FEATURES OF A MALIGNANT MEDULLARY HYPERNEPHROMA OF THE KIDNEY.[1]

JOSEPH C. OHLMACHER, M.D.

(*Fellow in the Department of Pathology, Northwestern University Medical School, Chicago.*)

This tumor, about the size of a large orange, growing from the superior extremity of the left kidney and encroaching upon the renal substance to occupy the upper two-thirds and hilus of the organ, was obtained from Mercy Hospital, Chicago, some two months ago. It was removed at operation by Dr. E. Wyllys Andrews, November 1, 1901, from Mrs. M., forty-nine years of age, who gave a history referable to the growth dating back five years, at which time the first attack of renal pain and hematuria was suffered. The tumor was discovered by the patient six months before operation. The immediate results of the operation were good and the patient was discharged from the hospital November 27. But she died December 15, metastases having appeared in the left temporal bone, right side of the neck, left axilla, left breast, right abdominal wall, and beneath the line of incision in the lumbar region. The left leg became swollen and edematous, presumably from venous thrombosis. No autopsy was secured.

The original tumor presents the usual anatomical appearance of a malignant hypernephroma, though the yellow color said to have been present in the fresh state has been lost in the formaldehyde-preserving process. While it pushes into the pelvis of the kidney so as to nearly obliterate it, the ureter is left intact. The renal vein, however, is invaded.

After a thorough histologic study of the neoplasm in which various methods were applied like the staining for fat, glycogen, reticulum, elastin, and dichromophilism of the nu-

[1] Read, in abstract, March 29, 1902, at the Second Annual Meeting of the American Association of Pathologists and Bacteriologists, Cleveland, O.

cleolus I have concluded that it is not only a genuine malignant hypernephroma, but one belonging to that more rare class of adrenal new growths which reproduce the *medulla* of the adrenal. That is to say, the larger portion of the primary tumor tissue is adrenal medullary substance, with here and there small islands of cortical cells.

Histology.

The following description is based on the histology of a typical section of this tumor. Its capsule is quite thick and is made up of a rather dense, slightly-nucleated fibrous tissue containing numerous threads of elastic tissue. It is not very vascular. Lying within it are well-defined nests of small, mononuclear cells with deeply staining nuclei and scanty cytoplasm. These same cells are seen, in large numbers, just beneath the capsule or extending into the deeper tissue. They will be described more in detail later. Near the center of one of the intra-capsular cell-nests is an epithelial-clad tubule. Others of these tubules are seen in the capsule and within a broad band of fibrous tissue which traverses the section at an oblique angle, they, however, being independent of the cell-nests. (See Plate XXXVIII., Fig. 5.) I am inclined to regard these as remnants of the Wolffian body on account of their striking resemblance to these embryonic structures. Persistent Wolffian tubules have recently been recognized in misplaced adrenal rests, but, so far as I can determine, have not been described in malignant hypernephromas.

Strands of fibrous tissue varying in thickness run at various angles from the capsule into the tumor beneath, there aiding in the formation of an anastomosing reticulum (staining prominently by Mallory's anilin-blue combination) which limits round, oval, or elongated alveolar spaces, filled with the characteristic cells. At the periphery of the tumor this inter-alveolar reticulum is less abundant and less dense than that found in the deeper portions, where it is present in large quantities and is so dense as to appear quite homogeneous; at the periphery there is also a closer anastomosis of the in-

ter-alveolar reticulum which here limits smaller alveoli, many of them being elongated. A fine, intra-alveolar reticulum is given off from the coarse, inter-alveolar reticulum, which, especially in the smaller alveoli, often forms a close meshwork supporting the constituent cells. The disposition of the coarse reticulum in this hypernephroma is precisely that of the medulla of the dog's adrenal obtained by the methods of Spalteholz or Mall as shown by Flint[1] in his excellent study of this organ. (See Plate XXXVIII., Figs. 1 and 2.) As to the presence of an intra-alveolar reticulum in the medulla of the normal adrenal there is still a dispute.

Blood-vessels with intact endothelium lie within the coarse reticulum. At the periphery of the tumor the lumina of these vessels are generally narrow, but as they penetrate more deeply into the tumor they widen into sinuses which, at first glance, appear simply as large spaces limited by fibrous tissue and surrounded by the alveolar cell-groups. Closer observation, however, shows that these spaces are lined with endothelium and that they often communicate with one another by vessels with much narrowed lumina. Blood-vessels in transverse section are of rather infrequent occurrence.

The proper tumor cells, filling the alveoli, are large and polyhedral, with large, faintly staining nuclei containing one or more nucleoli, and a scanty amount of finely granular, poorly-staining, vacuolated cytoplasm. The nucleoli show a tendency to stain with the orange, while the nuclear chromatin takes the blue with Mallory's reticulum stain (dichromophilism of the nucleolus). So far as I can determine, these cells do not rest directly on the endothelium of the blood-vessel, as is the case in hypernephromas of cortical origin. The cells are arranged in a sort of a rosette about the blood-vessels which are cut across transversely just as is seen in the normal adrenal medulla. The character and arrangement of the cells also bears a marked resemblance to the adrenal

[1] The Blood-Vessels, Angiogenesis, Organogenesis, Reticulum and Histology of the Adrenal. Contributions to the Science of Medicine by the pupils of William H. Welch, 1900, pp. 153–228.

medulla of the dog which Flint shows to be the same as that in the adrenal medulla of the higher animals including man.

Islands of cells from the adrenal cortex are seen lying among the medullary cell-groups surrounded by fibrous tissue, lying within the veins or blood-sinuses, or, in sections from certain parts of the tumor, entirely within the capsule. These cells vary in size, being generally quite large, with one or more large vesicular nuclei and one or several nucleoli. Their cytoplasm is abundant and has a marked affinity for acid dyes. Some of these cells are high-cylindric in type, others are round or polyhedral. Evidence of mitosis is occasionally seen in them. Some of the high-cylindric cells are arranged in the form of tubules or in the shape of the letter S or U, probably depending upon their position in the tissue when sectioned. (See Plate XXXVIII., Fig. 3.) These structures are regarded by Kelly[1] as the analogue of the adrenal cortex of man and as indicating a reversion to a lower vertebrate type (birds, for instance). Some of these structures when compared side by side with the tubular-like structures of the zona glomerulosa of the dog's or horse's adrenal show a striking resemblance (See Plate XXXVIII., Fig. 4), and I am strongly impressed, therefore, with the correctness of Kelly's view.

The small, round cells already referred to as appearing in groups within the capsule and beneath it will bear further description. Many of the cells beneath the capsule are arranged in rows more or less parallel with it or along the sides of blood-vessels as they penetrate into the deeper portions of the tumor. Some are seen in the blood-vessels or in rather large, rounded groups near the periphery of the tumor substance. Some of the larger, rounded groups are subdivided into smaller groups by a fine reticulum, and some are apparently surrounded by small capillaries. Fine capillaries are present in many of the groups. As some of these small cell-groups extend into the tissue beneath, a gradual transition into the mature, medullary cell-groups can be noted. In the light of the researches of Flint on the adrenal I have come

[1] On Hypernephromas of the Kidney. Philadelphia Medical Journal, July 30, 1898.

to regard these cells as immature or embryonal medullary cells because of the similarity in their form and arrangement with these elements in the developing adrenal.

The only place that smooth muscle is found is just beneath the capsule in sections from a certain part of the tumor. Elastic fibers are found in the capsule, in a broad band of fibrous tissue traversing the whole section at an oblique angle, and as fine threads lying among groups of embryonal medullary cells. Areas of necrosis are scattered through the tumor, and large and small foci of hemorrhage are abundant. Numerous phagocytes containing a yellowish-brown pigment lie among the red blood-corpuscles. A yellowish-brown pigment is seen scattered throughout the specimen, some of it being apparently within the medullary as well as the cortical cells, but a large part of it is seemingly lying on the cells. Some of this pigment, together with that in the phagocytes, answers to the iron test. Henle's reaction could not be obtained because of the formaldehyde-fixing process through which the tumor had passed. The tests for fat and glycogen are positive.

Small foci of tumor substance within the kidney parenchyma show practically the same histologic structure as does the bulk of the tumor. The growth into the renal vein, on the other hand, is made up of cortical cells which resemble those adrenal tumors which reproduce the zona fasciculata. The fact that all of the tumor cells found in the blood vessels are cortical cells, and that the growth into the renal vein is composed entirely of such cells, would seem to warrant the suggestion that all metastases through the venous system should be made up of adrenal cortex, and this despite the fact that the bulk of the primary tumor reproduces the adrenal medulla. That islands of cortical and medullary cells are occasionally found entirely within the capsule of this tumor is also very suggestive, for were such islands to continue growing they would produce nodules reproducing the adrenal cortex or medulla, as the case might be. One nodule at the uppermost part of the tumor under consideration is, apparently, entirely separated from the main tumor by a

broad band of fibrous tissue. Though it resembles the bulk of the tumor histologically, its isolation would indicate that it had developed from one of the intra-capsular groups of medullary cells. This, at least, suggests the desirability of examining all parts of a hypernephroma before deciding its histogenetic nature and of basing the diagnosis upon the findings in the main mass of the primary tumor. Certainly a diagnosis based upon appearances shown in the vascular metastases or in the accessory nodules might be misleading.

Conclusion.

The reasons for ascribing a medullary origin to this adrenal tumor and its principal histogenetic features may be reviewed as follows:

1. The disposition of the reticulum which, by anastomosis, forms round, oval, or elongated alveolar spaces filled with the characteristic cells, is peculiar. The arrangement of this reticulum is precisely that of the adrenal medulla in the dog.

2. The character of the component cells, which are large and polyhedral, with large, faintly-staining nuclei, and a scanty, poorly-staining, vacuolated cytoplasm, is identical with that of adrenal medullary cells.

3. The widening of the venules and blood sinuses as deeper portions of the tumor are reached is similar to that shown by the adrenal medulla.

4. The presence of embryonic or immature medullary cells beneath the capsule of the tumor and from here penetrating into the deeper portions, at the same time undergoing a transition, is like that seen in the developing normal adrenal.

5. Another of the interesting histogenic features of this tumor concerns the occasional islands of cylindrical cortical cells which take a distinct glomerular arrangement like that seen in the zona glomerulosa of the horse or dog and which are regarded by Kelly as indicating a reversion to a lower vertebrate type.

FIG. 1.

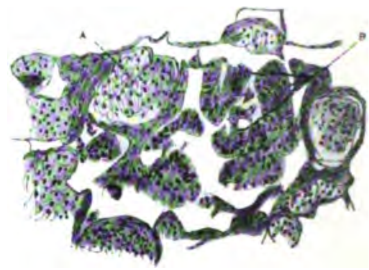


FIG. 3.

FIG. 4.

FIG. 2.

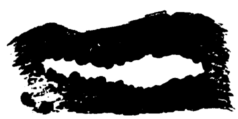


FIG. 5.

6. In the capsule of the tumor peculiar epithelial-clad tubules are occasionally seen which are regarded as remnants of the Wolffian body.

7. The possibility of mistaking the histogenetic nature of the primary tumor from the structure of accessory nodules or from metastases is also indicated.

EXPLANATION OF PLATE XXXVIII.

ACCOMPANYING DR. JOSEPH C. OHLMACHER'S ARTICLE ON MALIGNANT MEDULLARY HYPERNEPHROMA.

Fig. 1. — (A) The coarse inter-alveolar reticulum of the medulla of the dog's adrenal obtained after the Spalteholz method. (B) Same after Mall's method. (Copied from Flint.)

Fig. 2. — The inter-alveolar reticulum of the malignant medullary hypernephroma, from a section stained by Mallory's method. The *X* indicates alveolar spaces occupied by the characteristic medullary cells. The other spaces are blood vascular ones. (Obj. $\frac{2}{3}$, e. p. 1, B. and L.)

Fig. 3. — A section of the primary tumor showing at A the predominating medullary structure, and at B the group of cylindrical cortical cells making U- and S-shaped tubules, lying in a blood sinus. (Obj. $\frac{2}{3}$, e. p. 1 B. and L.)

Fig. 4. — (A) The tubule-like cortical cell group seen in Fig. 3, more highly magnified. (B) Similar structure from zona glomerulosa of a dog's adrenal. (Obj. $\frac{1}{8}$, e. p. 1, B. and L.)

Fig. 5. — One of the tubules in the capsule of the tumor resembling the tubules of the Wolffian body. (Obj. $\frac{1}{8}$,. e. p. 1, B. and L.)

A PRELIMINARY NOTE UPON CERTAIN MECHANICAL MICROTECHNICAL FACTORS CONCERNED IN PRODUCING SEGMENTATION AND FRAGMENTATION OF THE MYOCARDIUM.[1]

WALTER H. BUHLIG, B.S.

(*From the Department of Pathology, Northwestern University Medical School, Chicago.*)

INTRODUCTION.

The keynote of the motive for this investigation is expressed by the ideas quoted in the following words from A. B. Lee:[2]

"Minute examination of paraffin sections sometimes reveals certain distortions and dislocations or even ruptures of delicate elements. I have often noticed that in certain regions of my sections all the karyokinetic figures are drawn up to one side (always the same side) of the nucleus, leaving the rest of the nucleus empty and vacuolar in appearance. The achromatic fibrils of the division spindle are frequently ruptured, and I have not rarely found isolated chromosomes lying far from the nucleus in the body of cells, or even outside the cells themselves. These phenomena have generally been ascribed to 'shrinkage' caused by the action of the fixing agents or the processes of dehydration or imbedding. Heidenhain (*Ueber Kern u. Protoplasma* in *Festschr. Herrn Geheimr. A. v. Kölliker gewidm.*, 1892; see *Zeit. f. wiss. Mik., IX.*, 2, 1892, p. 200) thinks that they are often caused by *excessive tilt* of the under surface of the microtome knife. If this be found to be the case, the knife should be readjusted by means of a piece of cardboard placed in the jaws of the clamp. I would suggest that another cause of these defects is to be found in the imperfec-

[1] Read March 29, 1902, at the Second Annual Meeting of the American Association of Pathologists and Bacteriologists, Cleveland, Ohio.

[2] A. B. Lee. The Microtomist's Vade-Mecum, Third Edition, Philadelphia, 1893, pp. 182–183.

tion of the edge of the knife. If the knife be blunt, or have a rounded or curled edge caused by untrue honing or stropping, it will, of course, act in respect to minute structures as a plough rather than a cutting instrument, and thus produce the appearances described."

The object of this study, which was undertaken at the suggestion and under the direction of Prof. A. P. Ohlmacher, was to determine in how far the microscopic appearances designated "Segmentation and Fragmentation of the Myocardium" were the result of artificial conditions. It was assumed that these appearances were in reality artifacts produced principally by certain mechanical factors. At the present stage of our work we are prepared to sustain this assumption in so far as it applies to many of the recorded examples of myocardial segmentation and fragmentation. It is not now proposed to make the sweeping assertion that all cases in this category are thus to be disposed of, though there is a possibility that further knowledge may take us so far as to conclude that a diagnosis of cardiac segmentation and fragmentation based upon the microscopic appearances of sections of tissues is erroneous in all cases. But the burden of this preliminary work is to indicate that this interesting subject must be reviewed and controlled along the lines which we shall outline here and endeavor to elaborate in the near future.

Material and Methods.

The material employed was that commonly used, including the apparently normal human heart and the evidently diseased human heart, the hearts of rabbits and dogs. The heart of a healthy dog treated in various ways was largely used, and with material from this single source a variety of pictures, from those of intact heart muscle to extensive segmentation and fragmentation, were produced.

Various methods of fixing the tissue were employed, like that by Zenker's fluid, Bensley's fluid, formalin solution, the mixture of Flemming, and Carnoy's chloroform-acetic acid-alcohol mixture.

Infiltration and imbedding were done both in celloidin and in paraffin of varying consistency. Several microtomes were used like the Automatic Precision Microtome of Minot (Bausch and Lomb), the Minot-Zimmerman Automatic Rotary Microtome, the Bausch and Lomb Student Microtome, and the large sliding microtome of Leitz. A number of different microtome knives were tried. Sections were handled in watch glasses (celloidin) and affixed to the slide (paraffin). Staining was mostly done by the ordinary hematoxylin-eosin method. The pyrogallol-osmic method and the reticulum stain of Mallory [1] were also used.

In the series of experiments thus far prosecuted it was found possible to reproduce most of the pictures usually described both as segmentation and fragmentation of the myocardium, the only exception being the most extreme examples of general segmentation seen in some human hearts. The so-called "focal segmentation" could invariably be produced under suitable conditions. Moreover, it was found possible to profoundly modify these pictures under varying artificial conditions. It will be impracticable to go into details here concerning the results. But the factors which apparently are concerned in producing these artifacts will be briefly considered. They are as follows:

Factors of Primary Importance.

(1.) *Imperfections of the Knife Edge.*—It is a fact well known to all working with microtome knives that even the knife looked upon as a very good one will show an irregular edge when examined under the microscope. With the low power these imperfections make themselves evident as a wavy line, which, under the high power, is exaggerated into a series of nicks of various sizes resembling saw teeth. To get such imperfection the knife need be neither blunt nor have a rounded or curled edge (see Lee, op. cit.), for with the very best honing and stropping no knife can be made to have

[1] The reticulum and elastic fiber stains will be further employed in the effort to discover how far dislocation of connective tissue fibrils coincides with breaks in the heart muscle elements.

an absolutely perfect microscopical edge. I believe the rough edge of the knife to be the active causative agent in the artificial production of segmentation and fragmentation, though there are factors which predispose the tissue to the tearing by the knife.

(2.) *Direction of Section.* — Going hand in hand with the rough edge of the knife is this second factor. By direction

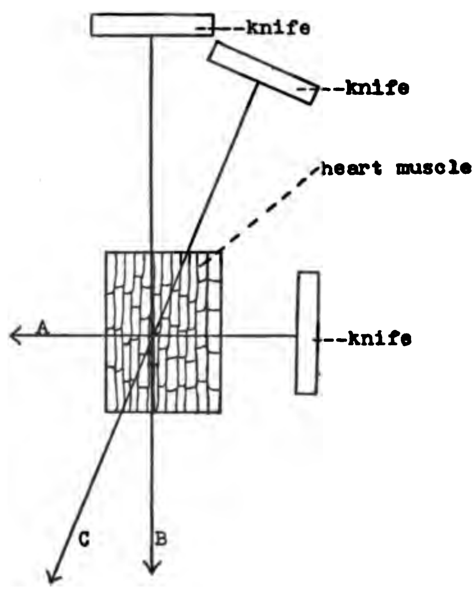


FIG. 1.

of section is meant the angle made by two lines: one represented by the direction of the cut, or, which is the same thing, the pull of the knife or the push of the object; and the other by the long axis of the heart muscle fibres. (See diagram.) In the sections cut for this work it was repeatedly observed that one direction of section was more favorable for the production of microscopic fracture than any other, and that one was at an acute angle, approximately 30°. (Direction C of diagram.) When sections were cut at a right angle to the long axis of the muscle cells (Direction A of diagram) the grooves made by the larger nicks in a knife

were more prominent than at any other angle; and though the muscular tissue in the region passed through by these nicks was badly shattered, the rest of the tissue seemed comparatively undisturbed. Even when cutting as nearly parallel as possible with the long axis of the muscle cells (Direction B of diagram), sections with less extensive segmentation and fragmentation were obtained.

In those sections cut nearly parallel with the long axis of the muscle cells, large breaks or fissures could be seen in the sections, besides the finer ones of segmentation and fragmentation. These larger breaks were of two kinds: (1) Those caused by nicks directly which could be traced through the section; and (2) which were most abundant and most important, those lying at right angles to the direction of the cut. These latter coarse breaks I believe to be caused by the pull of the knife, and to represent on a large scale what segmentation and fragmentation is on a smaller scale.

When the tissue was cut at right angles to the long axis of the muscle cells, it was noticed that where the grooves could be traced through the sections the muscle cells had undergone a lateral separation, such as is observed in all sections of acknowledged segmentation and fragmentation; and I therefore believe that the knife cutting at an acute angle represents a force which is the resultant of two forces, one acting at right angles to the long axis of the fibers separating them, and the other acting parallel with the long axis of the fibers pulling them apart.

In the tissue fixed in formalin, cement lines of all sizes were seen in the sections cut with the direction of section at an acute angle. When the tissue was cut at a right angle to the long axis of the cell, fewer cement lines were seen, as was also the case in the same kind of tissue cut in celloidin.

That brings up the question of cement lines. The difference in the number of cement lines when the tissue was cut in different directions was striking, but still more interesting was the position, size, and shape of these cement lines. Some extended over two cells; some showed a step-like arrangement within one cell; some extended from one cell laterally

for some distance beyond the muscle cell itself into spaces between the muscle fibers. Excluding the material fixed in Flemming, which shows these lines very well, none of the other tissues used in this work showed so many of these lines. It seemed that where the tissue in a section was more compact, that is, less broken up, there were more cement lines visible than in the badly torn regions. It may be that cement lines only represent the edge of small breaks where the cells are barely loosened. The sections I have cut strongly arouse the suspicion that these cement lines are artifacts.

Factors Probably of Secondary Importance.

(1.) *Reagents used in treating the Tissue.* — The tissue fixed in Carnoy's mixture [1] seemed especially suitable for producing the phenomenon of segmentation and fragmentation.

(2.) *Nature and Consistency of Imbedding Mass.*— Regarding the relative importance of celloidin, hard and soft paraffin as factors, little can be said at present. The sections cut thus far have given no constant results.

(3.) *Knife and Object Vibration.*— In working with material fixed in Carnoy's fluid, some curious dark spots were observed arranged transversely to the long axis of the heart cells. These spots cover the whole width and sometimes more than the width of a cell. They lie just at right angles to the direction of the cut. These spots look like accumulations of heart muscle fragments, which might have been pushed and piled up, for the areas between these transverse spots are devoid of tissue or are very thin and stain lightly. These striations strongly suggest vibration of the knife or object, or both, and seem in some places very closely to simulate cement lines.

(4.) *Knife Tilt.* — With the knife tilted somewhat more than is usual, the sections cut less readily and seem more broken up. The full significance of this factor has not yet been determined.

[1] Dr. S. H. Champlin, of Chicago, has suggested to us that heart tissue treated with chloroform is particularly fragile and in condition for artificial breaking. Possibly, too, the acetic acid of certain fixing fluids is a factor of similar effect.

(5.) *Thickness of Section.*— Thin sections are more favorable for the appearance of segmentation and fragmentation for two reasons: (1) There will be less tissue to hold together; (2) small imperfections in the sections will be more easily perceived.

(6.) *Knife Slant.*— What the influence of the slant of the knife is cannot now be decided. It is certainly true that paraffin sections of myocardial tissue cut with greater ease when the knife is set obliquely, but nothing conclusive regarding its importance in the production of segmentation and fragmentation can yet be reported.

Suspected Factors which may Apparently be Excluded.

(1.) *Kind of Knife.*— Several different knives, all in good cutting condition, were used and no striking difference in the sections could be perceived.

(2.) *Section Affixing and Mounting.* — The most of the paraffin sections were affixed to the slide by the water-albumen method. Some were flattened with the finger instead of with water. A number of observations upon the relative breaking by these two procedures were made and no difference in the appearances of the sections could be noted.

The dropping on of balsam and of the cover glass made no apparent difference in the sections.

(3.) *Rapidity of Section.*— It made no difference in the rupturing of the muscle fibers whether the sections were cut slowly or whether they were chopped off.

THE CHANGES PRODUCED IN THE HEMOLYMPH GLANDS OF THE SHEEP AND GOAT BY SPLENECTOMY, HEMOLYTIC POISONS, AND HEMORRHAGE.[1]

ALDRED SCOTT WARTHIN, M.D., PH.D.

(*Assistant Professor of Pathology, University of Michigan, Ann Arbor.*)

(*PART I.—SPLENECTOMY.*)

In the preliminary report of a study of the histology and pathology of the human hemolymph glands (Journal of Medical Research, July, 1901), the hypothesis was advanced that these glands under certain conditions may compensate for the spleen. This theory was based partly upon the general similarity in structure of hemolymph nodes and spleen, their common function of hematolysis, and the occurrence of transition-forms resembling accessory spleens, but chiefly upon the autopsy findings in a case of splenic anemia in which there was throughout the mesentery fat a new-formation of hemolymph nodes resembling splenic tissue. In other forms of anemia hyperplastic hemolymph nodes showing greatly increased hemolysis were also found. The evidence afforded by all these points was so suggestive of the existence of such a compensatory process that investigation along experimental lines was at once undertaken, with a view to the definite solution of the problem. Though the resemblance of the hemolymph nodes to the spleen in both structure and function had been noted by Vincent and Harrison, and later by Drummond, the present paper records the first experimental research regarding the nature and function of these organs. (See note.)

The anatomical changes following splenectomy have been studied by numerous observers, but the results of these investigations are greatly at variance. This is particularly the

[1] Read March 29, 1902, at the Second Annual Meeting of the American Association of Pathologists and Bacteriologists, Cleveland, Ohio.

case with reference to the question of total or partial regeneration of the spleen, and the occurrence of changes in the lymph nodes and bone-marrow interpretable as compensatory in nature. As early as 1680, Zambeccari observed in the mesentery of a dog four months after splenectomy numerous newly-formed nodules, isolated or collected into groups, of a yellow color, resembling lymphatic nodes.

According to Lussana, Gerlach and Eberhardt were said to have observed complete reproduction of the spleen in the frog after total extirpation. Beclard, Verga, and Legros experimented with rats and dogs, and denied the possibility of such a regeneration after splenectomy. Legros disputed further the possibility of any compensation on the part of the thyroid, omentum, or mesenteric glands.

In 1859, Philipeaux performed three total extirpations in white rats, and found eighteen months after a new-formation of splenic tissue at the site of the original organ. In 1861 Peyrani, after a number of experiments, attacked Philipeaux's conclusions as erroneous. The latter repeated his investigations, using young rats and rabbits, and was unable to find any evidences of regeneration. Explaining his first results as due to the fact that portions of splenic tissue had been left attached to the splenic vessels, he repeated his experiments with young rabbits, removing all but a small portion of the spleen. On autopsy complete regeneration of the organ was found, the new tissue presenting the appearance of normal splenic tissue. Peyrani carrying out further investigations again attacked Philipeaux's work and denied the possibility of any form of regeneration.

In 1880, Tizzoni and Fileti observed in splenectomized dogs an increase in size of the retroperitoneal and thoracic lymph nodes, the enlarged glands being of a red color. They attributed these changes to a post-operative lymphadenitis. In the same cases there was found a transformation of the fatty marrow of the long bones into red marrow. In two cases they found a new-formation of spleen-like nodules in the great omentum, in one old dog fifty-four days after splenectomy, and in one young one three and one-half

months after. The newly-formed nodules showed all stages of development, and from the appearances presented Tizzoni and Fileti believed the new splenic tissue to be formed directly from the adipose tissue by a process of absorption of the fat, conversion of the fat cells into reticulum, followed by a leucocyte infiltration and an active proliferation of the endothelium in the neighborhood of the small arterioles, leading to the formation of Malpighian corpuscles. Around these the continued proliferation of the reticulum and endothelial cells produced a pulp-like tissue, between the cells of which there was an extravasation of red blood cells. Around the whole a connective tissue capsule was formed.

In 1882, Tizzoni published his work upon accessory spleens and the new-formation of splenic tissue following pathological processes of the primitive organ. In numerous cases of indurative splenitis in the dog he observed a new formation of splenic nodules in the gastro-splenic ligament, but rarely in the great omentum. On the other hand, in cases of splenectomy he found numerous newly-formed lymphoid nodules throughout the subperitoneal fat, over the diaphragm, and in the pelvic, sterno-abdominal, and subcutaneous adipose tissue. In the splenitis cases the new tissue resembled splenic pulp, containing occasional groups of more closely massed leucocytes suggesting to a certain extent the Malpighian corpuscles. In the splenectomy cases the new tissue was for the most part provided with Malpighian corpuscles and a distinct capsule. In the new splenic tissue formed after splenectomy, he found nucleated red cells; these were not present in the newly-formed lymphoid nodules in the cases of indurative splenitis.

Winogradow, in the same year, reported observations of the anatomical and hematological changes occurring in splenectomized dogs. In one animal killed one hundred and thirty-two days after total extirpation of the spleen, the cervical, axillary, inguinal, and mesenteric glands were enlarged, soft, moist, dark, or bright red on section, the cortical portions particularly resembling spleen tissue. The color he found on microscopical examination to be due to great

numbers of red blood cells lying in the reticular spaces and filling up the lymph sinuses between the follicles and lymphoid cords, and also partly filling the peripheral sinus. Evidences of hemolysis were present, finely granular brown pigment being found in the sinuses and in the swollen cells of the reticulum. Winogradow regarded the finding to be similar to the appearances described by Tizzoni. In two animals killed, five hundred and seventeen and seven hundred and sixty days respectively after splenectomy, the same changes were found in all of the lymph glands, with the difference that they were firmer, their surface more irregular, and color brownish-red. The number of red blood cells in the sinuses was not so great, but the amount of pigment greater, the pigment being present not only in the sinuses, but also in the lymphoid tissue. The trabeculæ showed further a thickening resulting from the formation of new connective tissue. The presence of blood-containing sinuses was explained by Winogradow as due probably to a diapedesis into the lymph sinuses and not caused by artificial hemorrhage. He regarded it as very probable that the diminution of red cells in splenectomized animals during the first year after the operation could be caused by the continued diapedesis and destruction of the red blood cells in the lymph sinuses. The gradual return of the blood count to the normal he considers good evidence that the processes of blood regeneration are not interfered with, but are probably increased. Further, Griffini in this same year observed in dogs a partial regeneration of the spleen after the removal of a portion of the organ.

Tizzoni's observations were opposed by Foa in 1883, who denied wholly any regeneration of the spleen in the dog after total splenectomy. He further affirmed that the newly-formed nodules described by Tizzoni were present before the operation, the differences in color, etc., being due to hyperemia or infarction, or other pathological changes, and that no compensatory function could be ascribed to them.

In the same year Zesas experimented with rabbits and found in these animals, one week after splenectomy, that the

mesenteric glands were slightly enlarged, but of normal color and consistence. In animals four weeks after splenectomy the liver was enlarged and hyperemic, the mesenteric and bronchial glands greatly swollen, on section dark red, hyperemic, and firmer in consistence. In other animals seventeen weeks after the operation, the liver was hypertrophic and hyperemic, the bronchial and mesenteric glands heavily pigmented and of hard consistency. The kidneys were also hyperemic and pigmented. Zesas cites further experimental work of Hegar and Simon upon cats; in three instances, enlargement and pigmentation of the mesenteric glands were found after splenectomy.

In 1884 Tizzoni made a series of total splenectomies on the rabbit, but could confirm no new formation of the spleen nor any evidences of compensation on the part of thyroid, thymus, and lymph nodes. In the same year Mosler found in a dog, ten months after splenectomy, numerous spleen-like nodules of the size of a pea to that of a bean, dark red in color, and on section closely resembling spleen pulp. They were scattered throughout the great and lesser omentum. Microscopically the nodules presented appearances identical with those in the cases of Tizzoni and Winogradow. Mosler, however, regarded them not as newly-formed splenic tissue, but as neoplasms, "hemorrhagic telangiectatic lymphoma." Hyperplasia of the lymphatic glands was not constantly present in these cases. Mosler concluded from his investigations, that following splenectomy, there is a compensatory action on the part of both lymph glands and bone marrow, the latter appearing to play an important rôle, in one case resembling that of leukemia. These changes are not constant; in one dog, killed eleven months after splenectomy, there was not the slightest trace of any alteration having taken place in any of the remaining lymphoid structures.

In 1886 Gibson found, in splenectomized dogs, enlarged mesenteric glands containing both nucleated and non-nucleated red blood cells in their sinuses. Eternoid, in 1888, found in a young fox, one hundred and sixty-one days after splenectomy, a splenic nodule in the omentum, and newly-

formed lymphoid nodules in the mesentery, the other lymph glands of the body being enlarged and of a brownish color. In 1894, Vulpius observed no enlargement of the lymph glands in splenectomized animals dying soon after the operation, or killed at the end of five months. Laudenbach, in 1895, found that in splenectomized dogs hyperplasia of the lymph glands did not occur constantly, and that signs of increased blood formation were present in the bone marrow only. Ceresole in the same year found in splenectomized rabbits no hyperplasia of the lymph nodes and no new formation of the marrow.

In the reported cases of splenectomy in man, enlargement of the lymph glands has been noticed but a few times. Schultz in 1856, observed enlargement of the axillary glands in a splenectomized woman twenty-two years of age. Of the one hundred and seventeen cases of splenectomy collected by Vulpius in 1894, enlargement of the lymph glands was noted only in three, though special attention had been paid in a number of cases to the possibility of such an occurrence. Ceci, in 1889, observed a temporary increase in the size of the tonsils and thyroid after splenectomy. Czerny, Kocher, Lennander, and Riegner saw temporary lymphatic enlargement after splenectomy, and in 1900, Bolton reported a case in which after splenectomy for traumatic rupture there was a general enlargement of all the lymphatic glands. On the other hand, Czerny, Billroth, Albert, Trendelenburg, and others have failed to observe any change in the lymph nodes, tonsils, or thyroid after successful splenectomy. No autopsy reports of such cases exist as yet. Of special interest is the case of Hodenpyl, reported in 1898, of the autopsy findings in congenital absence of the spleen. Enlargement of all the lymph nodes of the body, axillary, cervical, bronchial, mesenteric, and the solitary follicles of the intestine was found with a new formation of lymphoid tissue in the adrenals and throughout the connective tissue of the liver. The case reported by Albrecht in 1896 of numerous small "accessory" spleens scattered throughout the peritoneum, the primary spleen being of the size of a small walnut, though

explained by this observer as due to some congenital disturbance of the spleen anlage, is open also to the interpretation of a compensatory process. A number of cases have been observed of the occurrence of very large accessory spleens, the enlargement being interpreted as compensatory in nature.

From this brief review of the literature it will be seen that none of these investigators was aware of the existence of modified lymph nodes constantly containing sinuses filled with blood, and to which the term "hemolymph gland" has been applied. It is, however, very evident that the descriptions given by Tizzoni, Winogradow, and Mosler of the new-formations found in the omentum and mesentery of splenectomized animals apply so closely to these glands that it seems reasonable to conclude that these structures were in reality hemolymph nodes. Mosler's description in particular applies to the hemolymph nodes and his diagnosis of "hemorrhagic telangiectatic lymphoma" is of peculiar interest.

The animal chosen for the investigations recorded in this paper was the sheep, for the reason that the hemolymph nodes in this animal are very numerous in the prevertebral fat and are easily found because of their size and distinctive dark-red color. Their normal histology has been very well worked out by Vincent and Harrison, and Drummond. To the work of these investigators my observations have added a number of new points of importance. The normal histology may be briefly condensed as follows:

OCCURRENCE. — The more careful the dissection the greater the number of hemolymph nodes found to be present in the animal; a rough calculation based upon animals examined at the slaughter-house makes the average occurrence between three and four hundred. They are most numerous in the prevertebral fat of the retroperitoneal, thoracic, and cervical regions, but are present also in the pelvic fat, along the iliac vessels, in the mesentery, omentum, anterior mediastinum, in

the fat about the anus, and very rarely on the diaphragm, in the subpleural tissue and in the axillary region.

Gross Appearances. — Their average size is that of a yellow mustard seed or a small pea, but all sizes are found from a pin-head up to a coffee-bean, and rarely as large as a cherry. The smallest ones are usually slightly flattened, the intermediate ones more round, while the larger ones are somewhat flattened. Numerous blood vessels pass to the node, in some cases surrounding it with a plexus, in other cases but few vessels are found. Some have lymph vessels, others apparently none. The larger nodes have a distinct hilum into which the vessels enter. The exact relations of the vessels and the mode of circulation have not as yet been made out. The surface of the node is usually smooth, but occasionally small elevations are present giving the node the appearance of a minute raspberry. The color is usually dark red, almost black; the nodes containing more lymphoid tissue and smaller blood sinuses are lighter, other nodes are of a brown or chocolate color. The consistence is soft and elastic, the delicate capsule is easily ruptured, the node then appearing as a small blood clot. On section the appearance of the nodes varies greatly, as the relative amount of lymphoid tissue to the size of the blood sinuses is hardly ever the same.

Smears. — The smears made from the blood contained in the sinuses of the dark-red glands resemble those made from peripheral blood. The proportion of leucocytes may or may not be greater. From the lighter-colored glands containing more lymphoid tissue, and from the chocolate-colored ones, it is difficult to obtain the blood unmixed with the tissue-cells. Surface smears contain a great variety of cell-forms, a detailed description of which must be omitted here. Phagocytes containing pigment or red cells are constantly present. No nucleated red cells were ever found.

Microscopical.— On microscopical examination the majority of the dark-red nodes present a thin capsule of connective tissue and unstriped muscle broken at intervals by blood vessels. Beneath the capsule there is a blood sinus of varying size from which similar sinuses run through the node in

all directions, separating the cords or islands of lymphoid tissue which may be relatively large or small. In some cases the node is almost entirely made up of blood sinuses. These are traversed by a reticulum of varying amount. A division into cortical and medullary portions may be made in the majority of the nodes, but the latter is much less. In the cortical portion, collections of cells resembling lymph follicles are usually present. In some nodes these are not found, the node consisting of delicate trabeculæ of lymphoid tissue traversing large blood sinuses and held together by delicate threads of reticulum. The reticulum is apparently lined by endothelial cells, in its meshes are always present large phagocytes containing blood-pigment and disintegrating red cells. In the brownish colored nodes these phagocytes are so numerous as to almost completely fill up the sinuses; at the same time there is apparently a thickening of the trabeculæ and reticulum and an increase of the lymphoid tissue, the nodes coming to resemble more closely the ordinary lymphatic glands. All possible stages of transition are found, the appearances suggesting a constant new formation of hemolymph nodes and their development into ordinary lymphatic glands, with a possible cyclical function of hemolysis. No evidences of blood-formation have been found under normal conditions. The thickness of the capsule and trabeculæ, the size of the blood sinuses, the amount of lymphoid tissue, and the amount of reticulum traversing the sinuses are not constant factors, hence the greatly varied microscopical pictures presented by the organs. A very striking and constant appearance is the great number of connective-tissue mast-cells found in the reticulum and stroma. Mononuclear eosinophiles are also numerous. In the smaller nodes containing but little lymphoid tissue the majority of the reticular cells may exhibit the mast-cell granulation; in the nodes where active hemolysis is present their number is not so great.

EXPERIMENTAL.—The animals operated upon were allowed to fast for several days preceding the operation, as experi-

ence showed in the case of one animal not so treated that the distended stomach greatly increased the difficulty of the operation. In the fasting animals the operation was performed with ease and without important loss of blood. Chloroform was used as an anesthetic, the animals not taking ether well. The abdomen was shaved before operation and asepsis carried out as far as possible. The animals were fastened to the operating table in the right-side position, this being found more favorable for the operation than the dorsal position. The incision was made in the left hypochondrium in the splenic region, about a hand-breadth below and parallel to the edge of the ribs. The operations were kindly performed for me by Drs. Peterson, Morley, Griffin, and McNamara, and I take this opportunity of acknowledging my indebtedness for the favor. All the sheep operated upon suffered severely from shock during the first twenty-four hours following the operation, fifty per cent of the cases dying from this cause within this time. The survivors reacted quickly and by the third day were apparently as well as before the operation. Because of the great mortality it was decided to make use of goats instead, the two operated upon for this series of experiments showing no symptoms of shock and recovering quickly. Inasmuch as the hemolymph nodes of the goat show practically the same distribution and structure as those of the sheep, it was decided to use the former animal in the remaining experiments. With the exception of local abdominal hernia developing at the seat of operation and the rapid development of a goitre in one of the goats no bad effects were observed to result from the operation. All the animals kept over a month after the operation showed a great increase of fat. The animals were killed by giving an anesthetic (chloroform), the abdomen being opened as soon as the animal was under the anesthetic. An opportunity was thus afforded of examining the glands while the blood was still circulating. On cutting through the diaphragm the thoracic glands were also examined before the heart had ceased to beat. The dissection was made while the tissues were still warm. All of the hemolymph

nodes and lymphatic glands discoverable were removed and fixed at once, in either mercuric chloride or Flemming's solution; the glands from the different regions were kept separate. In addition, portions of the other organs were taken for microscopical examination, smears of the glands and bone-marrow were made, and portions of the latter fixed for special examination. The material fixed in mercuric chloride was embedded in paraffin, that fixed in Flemming's in celloidin. Sections were made from each block; hematoxylin and eosin, kresylechtviolett, the triacid stain, the iron reaction, and borax carmine were the staining methods commonly used.

EXPERIMENT ONE.

SHEEP I. — Two-year-old merino grade wether. Splenectomy. Autopsy, twenty-four hours after operation.

Autopsy Notes. — Wound negative. Peritoneum in neighborhood of incision slightly injected, roughened and cloudy. No hemorrhage from splenic vessels. Passive congestion of all organs, particularly marked in the case of liver and kidneys. The hemolymph nodes are unusually large and prominent, all dark red in color. Lymphatic glands, particularly the mesenteric and retroperitoneal, are enlarged, hyperemic, and softened. The glands at the brim of the pelvis, along the common iliacs, are particularly enlarged and of soft consistency, but are of lighter color. A few of the retroperitoneal glands appear hemorrhagic.

Microscopical. — The blood-sinuses in the hemolymph glands are greatly distended, the cords of lymphoid tissue being apparently smaller and more widely separated. Very few lymphoid follicles are found in them, but small groups of more closely-packed lymphoid cells having a lighter central portion suggest their location. There is an apparent loss of leucocytes, the lymphoid tissue appearing poorer in cells. The mast-cells are greatly diminished in number, those present appear larger than normal, more round as if swollen, their granules staining both reddish and blue with kresyl violet. In some cells there is no distinct granulation,

the cell protoplasm staining diffusely reddish. There is no evidence of increased hemolysis. The lymphatic glands show extreme congestion, the veins of the glands being greatly dilated. Hemorrhages are present in many, the extravasation as a rule taking place into the stroma of the lymphoid tissue and not into the lymph sinuses. The lymph sinuses and the lymph vessels are also greatly distended. Blood cells in increased amount are found in these. The central portion of the follicles is in the majority of cases lighter than normal. The mast-cells in the lymphatic glands show changes similar to those in the hemolymph nodes.

EXPERIMENT TWO.

SHEEP II. — Three-year-old registered Shropshire ram. Splenectomy. Autopsy three days after operation.

Autopsy Notes. — Wound negative. Slight injection of peritoneum near incision. The stomach adherent to abdominal wall in neighborhood of operation. Adhesions easily separated. The liver is moderately hyperemic. The hemolymph glands are very prominent, but otherwise appear unchanged. The lymphatic glands are enlarged, softer, pinker than normal, the medullary portion of many showing a brownish tint.

Microscopical. — The sinuses are less distended with blood than in the case of Sheep I. The most striking change is the light appearance of the lymphoid follicles, due to a diminished number of cells and to a vacuolization of those present. The follicles are prominent because of their lighter staining and not because of the reverse, as is the normal case. They are smaller, there is an actual loss of cells, the central portion or even the entire follicle being formed of rather large branching cells with swollen vacuolated protoplasm. Some of the cells contain very large vacuoles. Either hydropic or fatty degeneration, or both, is present. This could not be definitely decided, as unfortunately the osmic acid in the Flemming's fixed specimens did not penetrate to the follicles. The large size and irregular shape of the vacuoles in many cases suggests hydropic change or edema rather

than fatty degeneration. The same change is present in the follicles of the lymphatic glands, but to a much less degree. The number of cells present in the follicles in all of the glands is evidently greatly diminished. In the blood sinuses of many of the hemolymph nodes there is an increased number of pigment-containing phagocytes and evidences of increased hemolysis. The lymph sinuses in the medullary portion of many of the lymphatic glands likewise are filled with pigment-containing phagocytes. The pigment in both cases for the greater part gives the iron reaction. The phagocytes which are stained diffusely brown with the pigment give an intense iron reaction, while those containing pigment granules show variation in this respect, some of the pigment granules giving the iron reaction, others not. These changes are more marked in the retroperitoneal glands than elsewhere, the thoracic glands coming next in point of change. The changes in the mast-cells of the hemolymph nodes are very striking. In some of the glands these cells appear as large, round, swollen cells containing coarse granules staining violet or blue with kresyl-violet. In other cells the granules are smaller and less distinct, while in other cases no distinct granulation can be made out, the cell protoplasm staining diffusely reddish or violet. Vacuoles are present in many of the mast cells. In one hemolymph node containing many cells showing mast-cell granulation, phagocytes having this granulation were seen, containing red cells in various stages of disintegration. There is also an increase of eosinophiles in many of the glands. In the anterior mediastinum several large deeply congested lymphatic glands as in Sheep I. were found. No nucleated red cells found in the blood of any of the glands or organs.

EXPERIMENT THREE.

SHEEP III. — Two-year-old merino ewe. Splenectomy. Autopsy five days after operation.

Autopsy Notes. — Wound negative save for a small stitch abscess. The stomach adherent to abdominal wall and diaphragm in the neighborhood of the operation; adhesions

easily separated. Stump of splenic vessels negative, no lymphoid tissue present in it. The hemolymph nodes not so prominent as in the normal animal; they are less red and more brown in color, but on the whole are increased in size. The lymphatic glands are enlarged, the cortex pale, the medullary portion pink or brown. Bone-marrow negative.

Microscopical. — The same changes are present as in Sheep II., but are much more marked. The light appearance and the diminution in size of the follicles of the hemolymph nodes due to vacuolization are very marked, less so in the follicles of the lymphatic glands. There is an increased number of the hemolymph nodes showing hemolysis, the number of phagocytes in the blood sinuses is everywhere increased, and there is a well-marked leucocytosis in the blood-sinuses and vessels. The lymph sinuses of the lymphatic glands also contain a greater number of pigmented phagocytes; as in Case II. the pigment in part gives an iron reaction, in part does not. The changes in the mast-cells are more marked than in Sheep II., and the number of eosinophiles greater. No evidences of blood regeneration found; no nucleated red blood cells found in any of the glands.

EXPERIMENT FOUR.

GOAT I. — Black female goat, two years old. Splenectomy. Autopsy two weeks after operation. (Animal developed large soft goitre during this period.)

Autopsy Notes. — Wound negative. Stomach adherent to diaphragm and abdominal wall in neighborhood of incision. No evidence of general peritonitis. No lymphoid tissue found in stump of splenic vessels. The liver somewhat browner than normal, not hyperemic. The hemolymph nodes are much diminished in number, particularly in the thoracic region, where they are apparently replaced by ordinary lymphatic glands of brownish color. The larger hemolymph nodes are paler than normal or are more heavily pigmented. Only a few of the larger ones containing large blood sinuses are found. On section, all the hemolymph

nodes show an unusual amount of lymphoid tissue. Numerous minute hemolymph nodes are found in unusual locations; they are apparently increased in number in the mesentery, pelvic fat, and cervical region. All lymphatic glands (retroperitoneal, mesenteric, thoracic, mediastinal, cervical, axillary, pelvic, inguinal, etc.,) are enlarged, softer than normal, many presenting small elevations on their surface. On section the glands show great hyperplasia of the cortex, the medullary portion being softer, more translucent, and of brown color. A number contain red streaks or spots corresponding to blood sinuses or vessels. In the thoracic prevertebral fat where hemolymph nodes are usually numerous, few of the latter were found, and large elongated masses of lymphoid tissue were present which on section showed a broad white cortex, and a brown translucent medulla. The superior mesenteric glands and the glands at the brim of the pelvis presented the greatest degree of enlargement and pigmentation, the peripheral glands much less. The bone-marrow showed no gross changes.

Microscopical.—The hemolymph nodes present hyperplasia of lymphoid tissue, in some cases nearly obliterating the sinuses. The follicles are for the greater part enlarged and form centers of lymphoid proliferation, masses of closely-packed lymphocytes surrounding the follicle, and extending into the blood sinuses, in some cases giving rise to an appearance suggesting splenic pulp. Numerous mitotic figures are present, especially at the periphery of the follicles. The mast-cells are diminished in number; numerous swollen ones are seen. Eosinophiles, particularly mononuclear ones, are found in abundance throughout the lymphoid tissue about the follicles, less commonly in the latter. There is also a proliferation of the reticulum thickening of the trabeculæ, and an increase in the number of the large mononuclear cells. Pigment-containing phagocytes are increased in number, and there is a great increase in the number of leucocytes present in the blood sinuses. The lymphatic glands present a great hyperplasia of lymphoid tissue, chiefly in the cortical portion, which is enlarged out of proportion to the medulla. The

elevations seen on the surface of some of the glands are due to hyperplastic follicles pushing out the capsule. All of the follicles are enlarged and apparently increased in number. Few show central vacuolization. Numerous mitotic figures are present, particularly about the periphery of the follicles. Throughout the lymphoid tissue between the follicles there are great numbers of eosinophilic cells. Localized collections containing hundreds of these are found scattered throughout the glands. These collections of eosinophilic cells are most numerous in the nodes showing the greatest degree of hemolysis; they lie usually in the zone between the cortical and medullary portions. The lymph sinuses of the medulla are distended and contain many pigment phagocytes. Transitional forms between hemolymph nodes and the lymphatic glands are numerous. In some of these the proliferation of the lymphoid tissue into the blood sinuses produces an appearance closely resembling splenic pulp. The connective tissue of the liver and lungs contains an increased number of wandering cells with localized lymphoid collections of small size. Numerous pigmented endothelial cells are found in the liver capillaries. There is a general leucocytosis. No nucleated red cells were found in any of the glands. Sections of thyroid showed dilated vessels and increased formation of colloid.

EXPERIMENT FIVE.

GOAT II.—Black male goat three years old. Splenectomy. Autopsy one month after operation.

Autopsy Notes.—Animal very fat. Thyroid somewhat enlarged. Laparotomy scar negative. Slight adhesion of stomach to abdominal wall. No evidences of general peritonitis. Liver somewhat browner than normal. No lymphoid tissue in stump of splenic vessels. Thymus greatly enlarged. The hemolymph nodes are apparently decreased in number and size in the regions where they are usually found in greatest abundance. Small ones are found in unusual locations, as the axilla and inguinal regions, and are very numerous in the mesentery, about the pancreas, and in the stomach and liver

ligaments. The largest ones are found in the pelvic fat. The small ones are dark red, the larger ones lighter colored or brown. All of the lymphatic glands of the body are greatly enlarged, particularly those of the mesenteric, posterior thoracic, and iliac regions. All show on section a great hyperplasia of the cortex with pigmented or reddish medulla. Numerous small glands are found resembling splenic tissue in whole or part. Bone-marrow negative.

Microscopical. — The changes in the lymphoid structures are of the same nature as those in Goat I., but are much more extensive. The hemolymph nodes from the usual locations show hyperplasia of lymphoid tissue at the expense of the blood-sinuses. In many cases the latter are almost completely obliterated, or so small that the tissue comes to resemble spleen pulp. The smaller nodes found throughout the mesentery and retroperitoneal fat resemble normal ones. The lymphatic glands present great hyperplasia of follicles and lymphoid tissue at the expense of the medulla. The glands resembling spleen tissue have the structure of lymph glands with medullary sinuses filled with blood; they are probably to be regarded as modified hemolymph nodes. In many of the glands red blood cells are found everywhere throughout the lymphoid tissue. No nucleated ones observed. The mast-cells are greatly decreased in number. Eosinophiles, mitotic figures, and pigmented phagocytes are numerous as in Goat I. The connective tissue of the liver and lung presents no greater infiltration than in that case. The number of pigmented endothelial cells in the liver capillaries is slightly greater; the cells of the convoluted tubules of the kidney also contain a small amount of hemosiderin. The sections of the thymus show great hyperplasia of lymphoid tissue with an apparent new formation of lymph follicles in the surrounding fat. In the adipose tissue of the retroperitoneal and pelvic regions small lobules of fat-cells are found whose capillaries are greatly congested and dilated. In some of these there is a lymphoid infiltration along the capillaries (early stage of hemolymph node?).

EXPERIMENT SIX.

SHEEP IV.—Two-year-old merino grade ewe. Splenectomy. Autopsy two months after operation.

Autopsy Notes. — Animal very fat. Laparotomy scar negative. Hernia of abdominal wall at scar. Stomach adherent to peritoneum at point of operation. Small purulent focus in wall near scar (stitch abscess). No evidences of general peritonitis. Thymus enlarged. Liver small and browner than normal. No hemolymph nodes found in thoracic or anterior mediastinal regions; numerous small ones, apparently newly-formed, scattered throughout the mesentery, subperitoneal fat, pelvic fat, around the rectum, and in the cervical, axillary, inguinal, and subcutaneous fat. With each one of the enlarged peripheral lymph glands there is a small hemolymph node lying in its hilum or close against its capsule. Those of the inguinal and axillary glands are as large as small peas. Along the vessels leading to one of the inguinal glands a number of hemolymph nodes are present. All of the lymphatic glands are greatly enlarged, particularly in the anterior and posterior mediastinal, mesenteric, and iliac regions. Along the brim of the pelvis and extending upwards along the abdominal aorta there is a double row of greatly enlarged glands, several of the size of a walnut. The superior mesenteric glands form a firm, solid cord about fifteen centimeters long and two centimeters in diameter. Similar elongated cords of lymphoid tissue are found in the anterior and posterior mediastinal regions. Many of the enlarged lymphatic nodes present the raspberry-like surface. On section the enlarged lymph glands present a greatly hyperplastic cortex, and a smaller deeply-pigmented medullary portion. Many of the nodes appear to possess no medullary portion, being composed entirely of white, soft, cortical substance. The raspberry-like elevations on the surface of some of the glands are apparently due to hyperplastic follicles pushing out the capsule. Transition-forms between hemolymph nodes and ordinary lymphatic glands resembling spleen tissue are common. In the subperitoneal

fat numerous minute red points are present. Small reddened areas are seen throughout the fatty marrow.

Microscopical. — The sections of the hemolymph nodes and lymphatic glands present the same lymphoid hyperplasia as in the last case, but to a more marked degree. The cortical portion is enlarged at the expense of the medulla. Many glands appear to be composed of cortical portion only, the follicles being greatly enlarged and apparently increased in number. In other glands the hyperplasia of lymphoid tissue is more uniform, all trace of the follicles being lost, and the medullary portion showing an extensive formation of lymphoid cords between the sinuses. In other nodes the lymphoid hyperplasia is still more marked, the distinction between cortex and medulla being entirely lost, and no follicles present, the sections presenting a uniform lymphoid hyperplasia resembling lymphosarcoma. In some glands the hyperplastic follicles are ten to twenty times the average size. Proliferation of the reticulum and thickening of the trabeculæ are also present in many glands. The decrease of mast cells, the numerous mitotic figures, eosinophiles, etc., are present as in the last case. There is undoubtedly a new-formation of hemolymph nodes out of adipose tissue. All stages of this development may be seen. The process begins with the angiectatic dilatation of the capillaries of a fat lobule, the fat cells of which become enlarged and lighter in color. At the same time the lobule becomes fairly well set off from the surrounding tissue by a thickening of its capsule. The next step is an infiltration of lymphocytes along the walls of the distended capillaries coincident with an absorption of some of the fat, the conversion of the fat-cells into reticular cells, and proliferation of the endothelium into the dilated capillaries, dividing them up into blood-sinuses. Continued lymphoid formation, development of sinuses, and absorption of fat lead to the fully-developed hemolymph nodes or ordinary lymphatic glands. If the blood-sinuses persist the structure of a hemolymph node is presented; if the formation of the lymphoid tissue is so great as to reduce the sinuses to capillaries the node assumes the structure of a lymphatic gland.

Transition-forms of all kinds are seen. In some cases the follicles are formed first, in others they are developed later in the lymphoid tissue. The evidences of greatly increased hemolysis in the hemolymph nodes and lymphatic glands are present. The dark color of the medullary portion of the latter is due to the great number of pigment-containing phagocytes present. Part of the pigment gives the iron reaction, part does not. Eosinophilic cells are most numerous in the glands showing the greatest amount of pigment. The sections of liver and lung show localized formation of lymphoid nodules in the connective tissue. There is a great increase of pigmented endothelial cells and also in the amount of the pigment contained in them. Apparently all the pigment in the liver gives the iron reaction. The cells of the convoluted tubules also contain small amounts of hemosiderin. In the liver the pigment is for the greater part in the peripheral zone of the lobule as in pernicious anemia, but in many lobules it is found in the central portion. In the long bones there is a beginning formation of red marrow. No nucleated red cells were observed either in the general circulation or in the hemolymph nodes or lymphatic glands. A general leucocytosis is present.

EXPERIMENT SEVEN.

SHEEP V. — Two-year-old merino grade ewe. Splenectomy. Autopsy two months after operation. A week before the animal was killed two doses of three grams each of toluylendiamin were injected into the large superficial veins of the hind legs. The animal gradually became very weak, on the third day could not stand. The red-blood cell count fell in one week from eleven millions to less than seven millions. The hind-quarters becoming paralyzed, the animal was killed.

Autopsy Notes. — Animal very fat. Skin, conjunctivæ, and fat of a decided brown tinge. Laparotomy scar negative. No evidences of general peritonitis. Necrosis of thigh muscles and venous thrombosis at points of injection. The hemolymph nodes show similar distribution as in Experi-

ment Six, and a similar new-formation in same regions, — one hemolymph node with each peripheral lymphatic gland. Numerous small hemolymph nodes are present behind the pancreas and along the splenic vessels. The color of all is much browner than in any of the other cases. The lymph glands show the same extensive hyperplasia, but on the whole are larger, softer, and much more heavily pigmented. One large gland at the brim of the pelvis is surrounded by a blood clot. Other small hemorrhagic glands are found in the retroperitoneal fat. The liver is not enlarged — soft and very brown. The kidneys are browner than normal.

Microscopical. — The microscopical appearances are similar to those in the preceding experiment, except for a great increase in the pigment and pigmented phagocytes found in the lymph glands and hemolymph nodes, liver, and kidney. A number of hemorrhagic glands are found. There is a similar new-formation of hemolymph nodes in the adipose tissues. The number of eosinophiles is greatest in the glands showing most extensive hemolysis. The destruction of the red cells seems to be restricted wholly to the sinuses of the hemolymph nodes and lymphatic glands, no phagocytes containing red cells being found in the liver or elsewhere. The bone-marrow is hyperemic with probable beginning lymphoid change. No normoblasts were observed in the general circulation or in the lymphoid structures.

EXPERIMENT EIGHT.

SHEEP VI. — Three-year-old merino grade ewe. Splenectomy. Autopsy five months after operation. Animal was very lean when first brought to the laboratory, but became very fat after the operation.

Autopsy Notes. — Animal very fat. Small stitch abscess in laparotomy scar. Stomach adherent to the abdominal wall in the neighborhood of the operation. No evidences of general peritonitis. The liver is small, brown, and hard. The kidneys are very brown. Fatty marrow reddened. The hemolymph nodes and the lymphatic glands show a greater degree of hyperplasia than in any of the preceding experiments.

Glands as large as walnuts are found along the brim of the pelvis and the abdominal aorta. The superior mesenteric gland is greatly enlarged, and there are long masses of lymphoid tissue in both anterior and posterior mediastinum. The cortical portion of the enlarged glands is white, the medullary portion deep brown. Many of the largest glands have the raspberry-like surface. On section the cortical portion is greatly hyperplastic, white, soft, and opaque; the medullary portions brown and more translucent. The enlarged follicles in many instances push the capsule outwards, forming the little elevations on the surface of the gland. In some glands no medullary portion is present, the cut surface presenting a homogeneous appearance. No hemolymph nodes are present in the thoracic region. There is a new-formation of these along the splenic vessels, about the pancreas, in the mesenteric, subperitoneal, and pelvic fat; and, in association with the peripheral lymph glands, one hemolymph node to each lymphatic gland.

Microscopical. — There is extensive hyperplasia of the lymphoid structures, new-formation of hemolymph nodes in the adipose tissues, and increased pigmentation. The lymphoid follicles are greatly increased in size and apparently in number. The glands showing a homogeneous surface present a uniform lymphoid hyperplasia with loss of structure, suggesting lymphoma or lymphosarcoma. The same transition-forms are found as in the preceding cases. The medullary portion of the majority of the glands shows thick lymphoid cords along the sinuses. The number of eosinophiles is very striking, the connective tissue trabeculæ of many of the glands containing them in great numbers. The localized collections of large numbers of eosinophiles are also present as in the other cases. These apparently bear some relation to the degree of hemolysis. In many glands proliferation of the stroma and thickening of the trabeculæ are very marked. Lymphoid nodules are present in the connective tissue of the liver and lungs. The endothelial cells of the liver capillaries contain a much greater amount of hemosiderin than in the preceding experiments. Moderate

hemosiderosis of the kidney is present. The bone-marrow is hyperemic and there is probably a new-formation of red marrow. No nucleated red cells were found in the general circulation or in the lymphoid nodes. There is a general increase of leucocytes.

BLOOD EXAMINATION.— The results of the blood study of the cases may be condensed as follows: The normal count of the red cells in the sheep was on the average twelve million five hundred thousand, the average leucocyte count about seven thousand. In the goat the red cells averaged about sixteen million, the leucocytes eight thousand. After splenectomy there was always a diminution in the number of red cells out of proportion to the amount of blood lost at the operation. The average decrease was two to three million. By the fifth month the number had increased, but was still lower than normal. No nucleated red cells were ever seen, and the red cells presented no morphological changes. The leucocytes in the peripheral blood were increased immediately after the operation, this increase during the first ten days being confined to the polymorphonuclear forms. After the tenth day there was an increase in the number of lymphocytes, the proportion gradually returning to the normal. At the ninth day the lymphocytes formed fifty per cent of the leucocytes. During the first few days following the operation the eosinophiles either disappeared entirely or were reduced to very small numbers, but after this time they showed a gradual increase. The number in the peripheral blood, however, was entirely out of proportion to the great numbers of eosinophiles observed in the lymph nodes. One of the most striking changes in the blood was the appearance of numerous atypical and degenerating leucocytes. These were most numerous during the first two weeks after splenectomy, but never entirely disappeared.

SUMMARY. — The changes following splenectomy, as shown by the above eight cases, may be briefly summarized as follows:

First Week. — Immediately following the operation there is an intense congestion of all the lymphoid structures (possibly due to shock?), a temporary decrease in the leucocytes in the vessels and sinuses of the lymph nodes, and changes in the mast cells. By the third and fifth days the congestion has greatly lessened, and the chief change is a vacuolization (hydropic or fatty degeneration) of the lymph follicles of both hemolymph and ordinary lymphatic nodes, and a withdrawal of leucocytes from these into the blood sinuses and vessels where there is a moderate leucocytosis. At the same time there are evidences of increased hemolysis, as shown by an increased number of pigment-containing phagocytes, increased number of eosinophiles, and a beginning proliferation of the lymphoid tissue of the hemolymph nodes.

End of Second Week. — Active proliferation of all lymphoid tissues had begun, as shown by the numerous mitoses present throughout hemolymph and lymphatic nodes. In the former the proliferation into the blood-sinuses gave them the appearance of being transformed into lymphatic nodes. Evidences of greatly increased hemolysis in hemolymph and lymphatic nodes was shown by large numbers of pigment-containing phagocytes; increase of eosinophiles and moderate leucocytosis were present.

One Month. — At the end of one month the proliferation of lymphoid tissue, hemolysis, eosinophilia, and transformation of hemolymph nodes into ordinary lymph glands were all more marked. In addition there was a new-formation of hemolymph nodes in adipose tissue.

Two Months. — At the end of the second month the findings were: advanced hyperplasia of all lymphoid structures, change of hemolymph nodes into lymphatic glands, new-formation of hemolymph nodes in the adipose tissues, increased hemolysis in all lymphoid structures, and marked formation of eosinophiles. Beginning pigmentation of the liver and kidneys, leucocytosis, and beginning new-formation of lymphoid marrow were also present.

Five Months. — At the end of the fifth month great hyperplasia and new-formation of lymph nodes, new-formation of

hemolymph nodes in adipose tissue, marked hemolysis, eosinophiles in the lymphoid tissues, pigmentation of the liver, and slight lymphoid change in the fatty marrow were the most important changes. The leucocytosis was less marked than at two months.

It is evident from the above that splenectomy in the sheep is followed during the first five months by a compensatory hyperplasia of the preëxisting lymphatic tissues, transformation of hemolymph nodes into ordinary lymphatic glands, and a new-formation of hemolymph nodes in the adipose tissues. No evidence of regeneration or new-formation of splenic tissue was found. The hyperplastic hemolymph nodes and lymphatic glands differ essentially from the spleen in the arterial relations of the follicles. In other respects the structure of the hyperplastic hemolymph nodes closely resembles splenic tissue both to the naked eye and microscopically. There can be no doubt that the new-formations observed in the dog after splenectomy, and regarded as "newly-formed splenic tissue" by Tizzoni, "hemorrhagic lymph glands" by Winogradow, and "hemorrhagic telangiectatic lymphoma" by Mosler, were either hyperplastic or newly-formed hemolymph nodes. Tizzoni's description of their formation in adipose tissue is confirmed in every detail by the finding in the above cases. His conclusions alone must be regarded as erroneous.

The establishment of the fact that hemolymph nodes may be formed directly from lobules of adipose tissue is of great importance in the light thrown upon many questions pertaining to the lymphoid tissues, particularly with regard to the relationship existing between the hemolymph nodes and the lymphatic glands. The new-formation of these structures repeats their embryonal development, and the earliest stages of development of both forms may run parallel. The hemolymph node must be regarded as a more primitive embryonal type than the lymphatic gland. It may develop into the latter through a further proliferation of reticulum and lymphoid cells. The first stage in the development of a

lymph node from adipose tissue is a dilatation of the small capillaries lying between the fat cells, and an infiltration of lymphocytes along their walls. If now the capillaries become angiectatic and converted into blood-sinuses through the formation of a reticulum arising from endothelial proliferation, the hemolymph node is formed; if on the other hand the capillaries remain small or undeveloped, and the lymphoid tissue increased at their expense while the lymph-vessels become enlarged and changed into sinuses, the structure is that of a lymphatic gland. The essential difference between the two is the degree of development of either blood capillary or lymph vessel into blood or lymph sinus. Intermediate stages of development produce the great variety of transition-forms found. The development of the hemolymph nodes in fat bears also a very striking resemblance to the development of red marrow out of fatty. In connection with the question of lymphoid conversion of fat it should be noted here that the work of Bayer on the regeneration of lymph glands in adipose tissue is also confirmed by the above results.

As to the question of the formation of red blood cells in the lymph glands after splenectomy as held by Gibson, Laudenbach, and others, no evidence of such a formation was found. In so far as the question of compensation for splenic function is concerned, the findings would indicate that hemolysis and leucocyte formation are the two functions which are taken up to an increased degree by the hemolymph nodes and lymph glands after splenectomy. That the splenic function is not perfectly compensated is shown by the disturbed equilibrium of the blood in the excess of hemolysis over blood-formation. This might be explained by the hypothesis that there is some hemolytic agent formed in the body which is normally taken care of by the spleen, or that the spleen in addition to a hemolytic function has an influence also in the new-formation of hemoglobin. Bottazzi's theory of the "hemocatatonistique" function of the spleen may be referred to in this connection. The beginning lymphoid change in the fatty bone-marrow in the second and fifth

months after splenectomy is to be regarded as compensatory only for the increased destruction of red blood cells, not for any abrogated splenic function of red cell formation.

Further, the vacuolization of the lymph follicles in the first few days after splenectomy might be taken as evidence of an intoxication by some substance drawing the leucocytes from the follicles so rapidly that degeneration of the cells of the germinal area results. The large number of degenerating leucocytes found in the circulating blood at this time may be also taken as evidence of this.

The great increase in the number of eosinophiles in the pigmented glands is also of interest as indicating a possible relationship between these cells and the destruction of hemoglobin. The pigmentation of the medullary portion of the lymphatic glands may be explained by the assumption that the pigment is carried from the neighboring hemolymph node to the lymphatic glands through the lymphatics. The new formation of a hemolymph node in the hilum or along the vessels of the peripheral glands, whose medullary portion showed pigmentation, might be taken in support of this view, — the hemolymph nodes acting as hemolytic organs passing the products of blood destruction on to the lymphatic glands for further elaboration. It is also possible that the pigmentation of the lymphatic glands is the result of a constant diapedesis of red cells into the lymph vessels and their destruction in these glands.

CONCLUSIONS. — 1. After total splenectomy in the sheep there is no evidence of regeneration of the primitive spleen or of the new-formation of splenic tissue.

2. The structural changes following splenectomy are: hyperplasia of existing lymphoid tissues, transformation of hemolymph nodes into ordinary lymphatic glands, and a new-formation of hemolymph nodes out of lobules of fat tissue, and a later proliferation of the red marrow.

3. There is no evidence of the formation of red blood cells in the lymph nodes after splenectomy.

4. The function of hemolysis is taken up first by the hemolymph node, later by the ordinary lymphatic glands.

5. The hemolytic function of the hemolymph nodes and hyperplastic lymph glands exceeds that of the primitive spleen, causing an excessive destruction of red cells. The resulting anemia is later compensated for by an increased activity on the part of the bone-marrow. It would appear, therefore, that the removal of the spleen leads to an increased production or retention of some hemolytic agent usually disposed of by the spleen. The effect of this hemolytic agent is either to stimulate the phagocytes in the hemolymph nodes to increased activity, or to so change the red cells that they are more easily destroyed by these phagocytes.

6. The presence of great numbers of eosinophiles in the glands showing great destruction of red cells seems to point to some relationship between these cells and

7. The appearances described by Tizzoni, Winogradow, Eternod, Griffini, and Mosler, as occurring after splenectomy are confirmed by this work, but given a different interpretation. Bayer's work upon the regeneration of lymph glands is also confirmed.

Note. — The above work had been entirely planned and largely carried out when the preliminary note by Morandi and Sisto (Contribution à l'étude des glandes hémolymphatiques chez l'homme et chez quelques mammifères, Archives ital. de Biologie, 1901) appeared. These observers studied the effects of splenectomy upon the hemolymph glands of dogs. They noted the increased hemolysis taking place in these structures, and also concluded that the splenic nodules of Tizzoni and others were hemolymph nodes.

BIBLIOGRAPHY.

Albert. Cited by Riegner.
Albrecht. Beiträg. z. path. Anat., 1896, Bd. 20, S. 513.
Bayer. Zeitsch. f. Heilkunde, 1885, Bd. vi.
Beclard. Cited by Tizzoni.
Billroth. Cited by Riegner.
Bolton. Annals of Surgery, 1900, Vol. xxxi, p. 734.
Bottazzi. Arch. ital. de Biol., xxiv, p. 462.
Ceci. Wiener klin. Wochensch., 1889, S. 424.
Ceresole. Beiträge z. path. Anat., 1895, Bd. 17.
Czerny. Cited by Riegner.
Drummond. Jour. of Anat. and Phys., 1900.
Eternod. Rev. med. de la Suisse rom., 1888.
Foa. Cent. f. klin. Med., 1884, Arch. ital. de Biol., iv, p. 299.
Gerlach and Eberhardt. Cited by Tizzoni.
Gibson. Jour. of Anat. and Phys., 1896, Vol. xx.
Griffini. Arch. ital. de Biol., 1883, Vol. 3. Arch. per le Scienze med., Vol. vi. Mem. dei prof. Griffini et Tizzoni, 1883.
Hodenpyl. N. Y. Med. Record, 1898, Vol. 54.
Kocher. Cited by Warbasse.
Laudenbach. Cent. f. Phys., 1895, ix, p. 1–4; Arch. f. path. Anat., 1895.
Legros. Cited by Tizzoni.
Lennander. Cited by Warbasse.
Morandi et Sisto. Arch. ital. de Biol., 1901, Vol. 35.
Mosler. Deutsch. med. Wochenschr., 1884, S. 337.
Peyrani. Cited by Ceresole.
Philipeaux. Cited by Ceresole.
Riegner. Berl. klin. Wochenschr., 1893, S. 177.
Schultz. Deutsche Klinik, 1856. Cited by Zesas.
Tizzoni. Arch. ital. de Biol., Vols. 1, 3, and 4. Atti della R. Accad. dei Lincei, Vol. xiii. Accad. reale dei Lincei, 1882. Arch. per le Scien. med., Vol. viii.
Tizzoni et Fileti. Atti della R. Accad. dei Lincei, Vol. x.
Trendelenburg. . Cited by Riegner.
Verga. Cited by Tizzoni.
Vincent and Harrison. Jour. of Anat. and Phys., 1897.
Vulpius. Beiträge z. klin. Chir., 1894, Bd. xi.
Warbasse. Annals of Surgery, 1894, Vol. 20.
Winogradow. Cent. f. d. med. Wissensch., 1882, No. 50.
Zambeccari. Cited by Ceresole.
Zesas. Arch. f. klin. Chir., 1883, Bd. 28, S. 157.

THE BLOOD VESSELS OF THE SUBMAXILLARY GLAND AND THEIR DEVELOPMENT.

JOSEPH MARSHALL FLINT, M.D.

(Professor of Anatomy in the University of California.)

(From the Hearst Anatomical Laboratory of the University of California.)

(Preliminary Note.)

The blood supply of solid organs in general may be divided into two types: those in which the main nutrient vessels enter at the hilus, and then break up and ramify in the substance of the gland, and those which derive their main supply from an arterial plexus in the capsule. After considerable ramification and extensive anastomosis, this plexus gives off vessels that penetrate into the substance of the gland. Organs with a blood supply of the first type are the kidney, liver, ovary, spleen, thymus, lymph, and salivary glands, while the adrenal, testis, thyroid, prostate, and pancreas are provided with a blood supply of the second type. Since the course of the arteries within an organ shows in general the lines along which it developed,[1] it would follow from the nature of these two types of blood supply that we have likewise two methods of organic growth. In one instance the development would be from the center outwards as in organs of the first class, and in the other from the capsule inwards as organs of the second type. We might term them instances of exogenous growth if the blood vessels develop from the capsule, and cases of endogenous development where the vascular supply enters the organ at the hilus and grows towards the capsule.

Excepting certain radical modifications that take place at the time when the embryo ceases to receive its nourishment from the maternal blood sinuses and independently undertakes the aëration of its own blood, it has been shown that

[1] Flint. The Blood Vessels, Angiogenesis, Organogenesis, Reticulum, and Histology of the Adrenal. Welch Festschrift, and the Reports of the Johns Hopkins Hospital, Vol. IX.

the conditions of the systemic circulation in embryos at term approximates very closely that in adult life. At the same time considerable less differentiation occurs in the intrinsic circulation of the individual organs than in the larger vessels of the general circulation. At the time of birth the structure of most organs is well developed, and the changes which take place are usually quantitative rather than qualitative. Accordingly the material for this research was obtained from the submaxillary gland of injected embryo pigs, and the results were subsequently shown to conform to the conditions found in the glands of dogs and human beings. Von Baer's old aphorism that embryology is the true lantern bearer for the study of organic forms, can have no more apt application than in the solution of the many puzzling and intricate relations found in the vascular supply of various organs. Certainly, developmental studies throw as much light on the problems of angiology as they do on those in other important fields of anatomy.

The well-known researches of Thoma on the histogenesis of the vascular system offers an explanation of some of the phenomena of vascular development, particularly in the relation between the velocity of the blood current and the size of the vessel that conducts it. The question of the ancestry of arteries and veins was solved by Thoma, who showed in chick embryos that originally they were always simple capillaries. Their subsequent transformation, according to this author, was due to their fortuitous location with reference to the primitive aortæ, and the venous ostia of the heart. It has been shown that these facts apply to the vascular development in mammals as well as the chick, and for vascular systems developing in three dimensions as well as those found in the area vasculosa where the vessels grow in two directions only. In considering the problems of angiogenesis in mammals it is apparent that Thoma's histo-mechanical principles do not suffice to explain all the facts, nor do they even entirely accord with them. The statement that a new growth of vessels follows a rise of blood pressure in the capillary area must be considered only as an hypothesis and not a

demonstrated fact, for this would make the vascular system the stimulus to the development of new cells, while there is considerable probability that it is the new cells which form the stimulus for the growth of new capillaries. It must be obvious that the principal factors that govern organic growth are resident in the cells rather than in the blood vessels, as is indicated by their behavior in the embryo before the vascular system is laid down. We have still much to learn concerning the factors that arrest the growth of organs when they reach the adult type, but there is little doubt that these phenomena are expressions of cellular rather than vascular activity, since the vascular system maintains far beyond the usual period of growth its power of progressive development. It is, so to speak, always in a state of unstable equilibrium, in which both progressive and regressive changes are possible. Certain facts in the development of the blood vessels of organs have already been demonstrated, the most important of which is that the intrinsic blood supply of organs marks out the paths along which the vasa comites of structure that compose it have developed. And by following the gradual increase in complexity through a series of injected embryos the succeeding changes from the simple embryonic to the adult form can be easily demonstrated. It is important to trace these changes, not only for the light they shed on the development of the vascular system, but because many obscure features in the structure of organs are elucidated when the mechanics of their development are known.

The evolution of the blood supply of the submaxillary can be readily followed in the injected submaxillary glands of a series of embryo pigs. As a number of investigators have shown, the first anlage of the submaxillary gland appears as a solid bud or projection from the buccal epithelium which grows backwards toward the angle of the mandible. This column soon branches and the branching end becomes surrounded by a fine capsule. The bud or column of cells forms the anlage of the dictus submaxillaris, while the branching end with its capsule represents the future gland in its simplest form. At a very early period this column of cells is ac-

companied by an artery, the branches of which form a fine plexus of capillaries about the growing column of cells. These unite into venules and then flow into the veins which accompany the artery. The growth of these columns is chiefly apical where the cells form little knobs. About these swellings a capillary network is also found.

In a pig eight centimeters long, nape breech measurement, the gland consists of a solid branching column of cells in a blastema formed of large, occasionally branching cell-elements. The arteries and veins at this stage run with the solid columns of cells which are now forming the future ducts of the gland. The arteries give off in their course little branches which form a capillary meshwork about the cell columns that extend to the apices. In a pig ten centimeters long the branching is increased somewhat in complexity, and the capillary plexus about the growing ducts is slightly denser.

In a pig at this stage the Aa principales, the Aa interlobulares, and the Aa sublobulares are already formed and the capillary meshwork around the growing apical buds represents all that in future stages will form the lobular circulation. This meshwork is composed of small arterioles which break up and form a fine network of spherical or ovoid spaces over the growing ends of the ducts. These unite again to form the venæ terminales. At this period the division of the gland into lobes is well marked as the region of the growing organ supplied by an interlobular artery is separated by a delicate septum of embryonic connective tissue from other similar adjacent parts of the organ. The ramification has advanced in a pig fifteen centimeters in length so that the intralobular vessels are already formed. The structure of the primitive lobule at this period, however, is very simple, inasmuch as the ducts and vessels branch only once or twice before ending in the terminal knobs, about which there is a capillary plexus that has all the characteristics of those already described in pigs of an earlier age. One notes that all arteries below those of the lobular type are accompanied by venæ comites. Lobular arteries and those which form

the intra-lobular system are accompanied only by single veins. The capillary plexus is somewhat denser than in the pig ten centimeters long and the vessels which compose it are slightly more regular in their outlines. The vein radicals originating in this network usually empty into the vein that accompanies the arterioles. Occasionally, however, they may unite and pour their contents into the veins in an adjacent alveolar system. In pigs fifteen, ten, and even eight centimeters in length the precipitate of silver nitrate shows the typical markings of the endothelium of the blood vessels. Arteries can be easily distinguished from veins in these specimens from the fact that more silver is deposited in them so that the former are black while the latter are a light brown. From the earliest stages one sees the plexuses of capillaries which surround the developing column of cells. These capillaries are derived from the arteries that accompany the cell-columns and the meshwork represents the primitive state of the plexuses which in adult life surround the secretory system of the whole gland. At first the meshwork is coarse and the capillary vessels composing it somewhat irregular, but as the pigs increase in age the plexus becomes more regular and some of its elements unite to form a larger venous plexus, which lies on top of the one formed by the anastomosing capillaries. Recapitulating, then, we see that the primitive blood supply of the sub-maxillary consists of a few branching arteries and veins that accompany simultaneously branching columns of cells which later form the ducts and alveoli. As the age of the embryo advances, this branching increases, until in a pig fifteen centimeters in length the beginning of the circulation of the lobule is established. This consists at this period of the terminal artery which later becomes the A. lobularis, a small capillary plexus, and a small venule. Obviously this seems to indicate that the ramification of the ducts within the lobule occurs after the pig has reached the age represented by a length of fifteen centimeters. It is about this time that the membrana limitans embraces the growing apices of the ducts, for one notes in stained preparations a slight gathering of the embryonic

connective tissue about these groups of cells that form the primitive lobule, and, at the same time, a diminution in the quantity of interlobular blastema. Anastomoses between the arteries are very rare, although they do sometimes occur. Anastomoses between the venæ comites following the arteries are very common, but those between veins of different systems are seldom seen, although they occur more frequently than between arteries.

In man the A. submaxillaris is a branch of the A. max. externa, which usually passes through a little sulcus on the surface of the gl. submaxillaris as it mounts up over the lower border of the ramus of the mandible. Sometimes, however, it may even be embedded in the substance of the gland. Occasional branches that supply the organ are also derived from the A. submentalis. The veins from the gland empty chiefly into the V. facialis communis and partly into the V. submentalis. In man, the arteries do not enter the hilus of the organ with the duct, but join the latter a short distance after it penetrates the gland and take up immediately the close relations with the duct which are observed throughout the developmental and adult period of life. The veins follow the arteries throughout their course. In the pig, the blood vessels join the ducts immediately after their entrance through the hilus of the organ before the ducts of the first order are given off. Since the relations between the ducts and blood vessels were so constant, it may be well to consider briefly the course of the ducts within the gland.

The distribution of the ducts in pigs is not unlike those in man, save that in the former it is somewhat more regular. In man the ductus submaxillaris enters the gland at the hilus and immediately breaks up into several ducts of the first order. These run a short distance before dividing into a secondary system, which ramifies extensively throughout the gland, and are termed interlobular ducts. From the latter are given off a set of sublobular ducts. These divide once or twice and then exhaust themselves in the lobular ducts which enter the lobules of the gland at the hilus. Once within the lobule the ramification is more extensive and the divisions of

these intralobular ducts radiate from the hilus toward the periphery of the lobule. They terminate in a short duct of smaller caliber termed the intercalary portion which connects them with secreting alveoli of the gland. The ducts are accompanied by the blood vessels and run in the thick processes of fasciculated connective tissue that form the interlobular spaces. These interlobular spaces have a certain resemblance to those of the liver, save that they have two veins instead of the single branch of the portal system found in the hepatic interspaces. Since the vessels have such and constant and important embryological relation to the ducts and the other structures of the organ, it is convenient in describing the vascular system to use the same terminology for the arteries and veins as well as the ducts.

The A. submaxillaris divides into the

(1.) Aa principales which break up into the
(2.) Aa interlobulares which break up into the
(3.) Aa sublobulares which break up into the
(4.) Aa lobulares which give off the
(5.) Aa intralobulares which divide into the
(6.) Aa terminales.

After forming a capillary plexus about the alveoli of the gland the elements of this plexus unite to form the

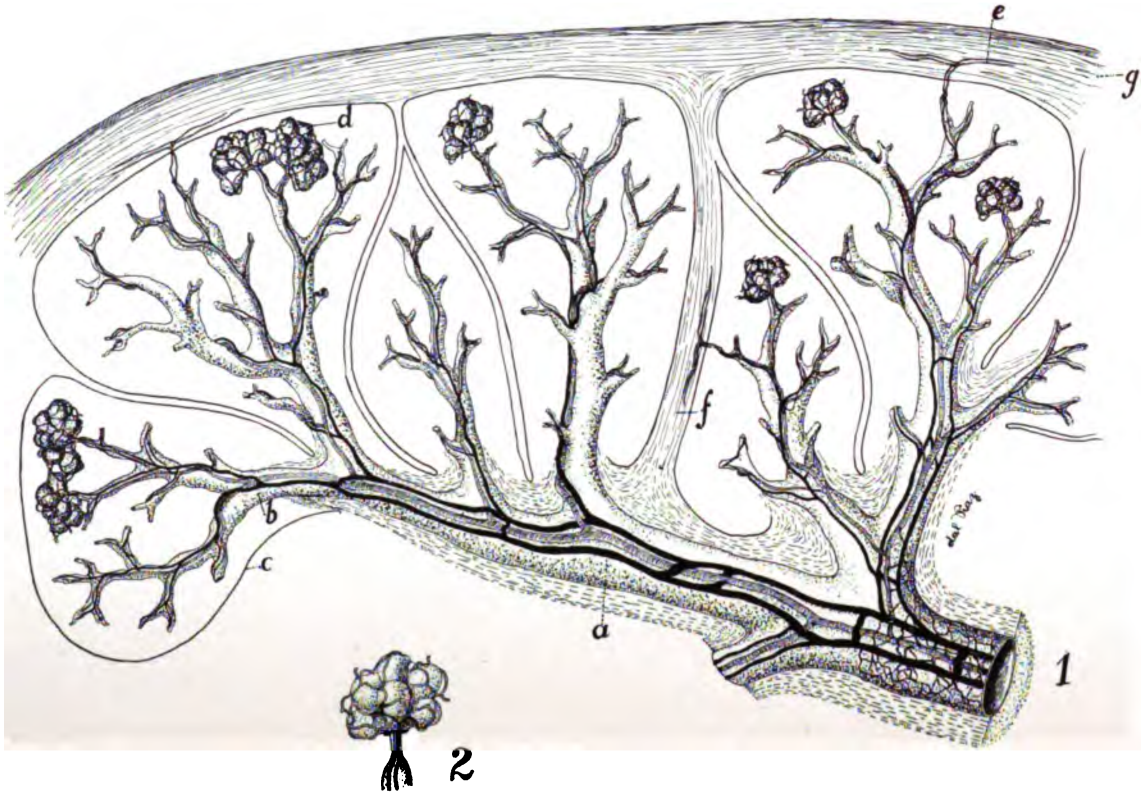
(1.) Vv terminales which join to form the
(2.) Vv intralobulares which join to form the
(3.) Vv lobulares which join to form the
(4.) Vv sublobulares which join to form the
(5.) Vv interlobulares which join to form the
(6.) Vv principales which join to form the
(7.) Vv submaxillares which usually empty into the vena facialis communis.

The submaxillary artery in embryo pigs enters the hilus of the gland at a short distance from the main duct and runs towards the latter until they converge and meet at the point where the ducts of the primary order are given off. After meeting the main duct, the A. submaxillaris immediately breaks up into the Aa principales and each of these branches

follows a duct of a similar order. The larger arteries and veins do not run very close to the lumen of the duct, but lie embedded in the connective tissue at the peripheral portion of the interspace which contains them. As the ducts and arteries ramify successively into the interlobular and sublobular vessels, this relation becomes somewhat more intimate. On the whole the course of the vessels is rather straight and the arteries do not wind or coil around the ducts, but simply branch with the latter, and the new branches pass with the new subdivisions of the duct. After dividing once or twice, the A. sublobularis finally enters the lobule at its hilus. It then ramifies within the substance of the lobule, the vessels maintaining in their intralobular course the same relations to the ducts that they held without the lobules. As the vessels divide within the lobule they radiate in three dimensions much like the branches of a small tree until they end in the ultimate arterioles that run with the intercalary ducts or terminals of the excretory system. These ultimate arterioles then break up into the capillaries which embrace the alveoli. The capillary meshwork is composed of ovoid or spherical spaces, and the capillary system of the whole lobule anastomoses throughout. In the capillaries embracing the alveoli arising from a given intercalary duct, small venules are formed which unite and run toward that duct as the venæ terminales. These now become the venæ comites of the Aa terminales. At the terminal intralobular ducts the terminal veins unite to form venæ intralobulares. The latter converge with their accompanying ducts and arteries and after uniting, pour their contents into the venæ lobulares, which leave the lobules at the hilus at the point where the ducts and arteries enter them. From the lobular artery to the lobular vein, this complete terminal system forms, in Ludwig's sense, the typical vascular unit of the submaxillary gland which has constant and definite relations to the lobules or units of structure, so that the blood supply of the whole organ may be said to be composed of a great number of these simple units together with the channels which carry the blood to and from them.

Veins from a number of lobules unite to form the venæ sublobulares. At this point the sublobular veins are doubled so that each artery and duct is accompanied by two veins instead of the single vessel that runs with them within the lobule. In the large interspaces these venæ comites have frequent anastomoses which are usually above, but sometimes below, the accompanying artery. At the termination of the sublobular interspaces the venæ sublobulares coalesce into the larger vessels which form the venæ interlobulares. These run with the accompanying interlobular ducts and arteries and finally unite to form the venæ principales, which are the largest veins found with the ducts within the gland. At this point the venæ principales fuse and form the main submaxillary veins. The latter leave the duct and then run with the submaxillary artery to empty usually into the venæ facialis communis as it passes over the glandula submaxillaris.

The walls of the ducts are supplied by small arterioles, given off at fairly regular intervals from arteries of the several orders. These smaller branches divide and form a dense plexus of capillaries situated just outside of the basement membrane, the meshwork of which is composed of rather elongated polygonal spaces. The longest diameter of these spaces runs at right angles to the long axis of the duct. In this plexus venous radicals are formed, which, by their coalescence, make a network of fairly large veins. This is situated just above the capillary plexus, and empties finally into the venæ comites of the ducts. These plexuses about the ducts are present in all branches and orders, from the beginning to the terminal system. Within the lobule the network is, however, much finer than in the extralobular system. The venous plexus about the intralobular portion of the excretory system is also much less developed. Around the intralobular ducts the blood supply consists of a single capillary network. The framework of the gland is supplied by a special series of arteries which are derived from the intralobular system. Branches of Aa intralobulares pass out, and after perforating the membrana limitans ramify in the substance of the capsule and the large septa which separate



FLINT.

BLOOD VESSELS IN SUBMAXILLARY GLAND.

the several lobes. These capsular and septal arteries are accompanied by veins which carry back the blood from the connective tissue of the septa and capsule into the intralobular systems. The capsule contains a comparatively rich plexus of vessels, some of the elements of which are derived from vessels in the periglandular connective tissue. Between this system and that of the gland proper there is a scanty anastomosis in the capsule through the agency of the capsular arteries. In the capsule the capillary plexus is coarse, and lies beneath the venous plexus, which is formed of rather fine small veins. These make up a regular polygonal meshwork composed of fairly large spaces. They are well shown in sections which cut the gland tangentially. As one looks down upon the lobule from above, in such a preparation the arteries and veins can, like the ducts, always be seen in the center of the lobule radiating out toward its periphery, but never quite reaching the limiting membrane. Capillaries alone seem to occupy that position, except in the instance where arteries leave the intralobular vessels to pass through the limiting membrane and ramify in the interlobular connective tissue, or in the substance of the capsule. A few vessels, other than those forming the plexus around the ducts, are often derived from arteries of the different orders to supply the connective tissue of the various interspaces.

TETANUS AND VACCINATION.[1] — AN ANALYTICAL STUDY OF NINETY-FIVE CASES OF THIS RARE COMPLICATION.

JOSEPH McFARLAND, M.D.

(*Professor of Pathology and Bacteriology in the Medico-Chirurgical College of Philadelphia.*)

During the year 1901 the occurrence of an unusual number of cases of small-pox in different parts of the United States stimulated the health authorities to a vigorous endeavor to protect the public by vaccination. Accordingly, during the year 1901, throughout many parts of the United States an unusual activity in vaccination took place.

The occurrence of a number of cases of tetanus succeeding vaccination, first in Cleveland, Ohio, and then in Camden, New Jersey, early attracted my attention, as this, to me, unknown complication seemed a matter of the greatest importance, increasing the danger of vaccination and correspondingly arousing the animosity of those misguided persons who have banded themselves together for the organized opposition of this well-recognized and only safeguard against small-pox.

The cases of tetanus in Camden have been so exploited in the newspapers and have been made the subject of so many editorials and comments in the medical press that they will, no doubt, form the starting point of numerous future attacks against vaccination, so that I deemed it important that as many cases of the complication as could possibly be collected be brought together and carefully analyzed, in order to determine whether tetanus be a necessary or an avoidable complication of vaccination. If necessary, we should be prepared for it and know how often to expect it; if avoidable, we should seek to eliminate it by every precaution regarding the selection of a superior virus and the performance of a careful operation.

[1] Read at the Second Annual Meeting of the American Association of Pathologists and Bacteriologists, March 28, 1902.

Tetanus is not a recognized Complication of Vaccination. — The only text-book I have been able to find make any mention of it is the recent edition of Osler's "Text-book of Medicine," in which it is simply stated that tetanus occasionally follows vaccination. In the "Minority Report" of the British Commission published in 1896, in which the disadvantages of vaccination are carefully and forcibly summarized, a single case, apparently the only one they were able to collect, is mentioned. Ordinary writers upon vaccination entirely ignore tetanus as a complication. The literature on the subject is extremely meager. In the Index Catalogue of the Library of the Surgeon General, but *seven* cases are mentioned under titles by which they can be recognized, and a total of fourteen cases are to be found in the literature. These occurred at somewhat remote periods in different parts of the country and were all attributed to secondary infection of the wound. It would seem reasonable, therefore, to conclude that tetanus has not been a frequent and important complication of vaccination in the past, either in this or any other country. It may, however, be surmised that what is true at the present time has also been true in the past, and that when tetanus occurred an attempt was made to suppress rather than publish it; but if in the past any such occurrence of tetanus had taken place as we have experienced recently, the medical literature would certainly contain some reference to it, as it does at present to the recent cases. For, suppressed as they are, very few cases having been published in detail, rumors of cases and editorials referring to cases and speculating upon their occurrence are to be found in many of the journals. The lack of published information concerning the complication cannot depend upon the failure to recognize it, as tetanus has been a well recognized disease for centuries, and indeed very little has been added to its symptomatology since the days of Hippocrates.

I have been able to collect a total of ninety-five cases. Throughout the paper I shall refer to *cases*, meaning by the term occurrences of tetanus that I have succeeded in authenticating and of which I have considerable detail, while

the term "*rumor*" will be used for cases of tetanus the occurrence of which seems certain, but concerning which, for various reasons, I have been unable to secure the details.

TABLE I.

Cases from the literature		14
Cases collected		
a. With complete details	40	
b. With incomplete details	13	
		53
Rumors		28
		95

Fatalities	61
Recoveries	24
Unknown	10
	95

Adults	25
Children	45
Unknown	25
	95

Males	38
Females	29
Unknown	28
	95

TABLE II.

Tetanus Cases — Showing Chronological Occurrence.

The following table shows the chronological order of the cases I have succeeded in collecting:

Chronology.	Cases.	Rumors.		
1854	1			
1878	1			
1882	3			
1886	1			
1889	1			
1891	1			
1892	0	6		
1893	1			
1897	1			
1898	3	2		
			January	0
1899	3		February	0
			March	1
			April	0
1900	1		May	0
			June	1
			July	1
1901	45	18 = 63	August	1
1902	5	1	September	1
Unknown date		1	October	1
	—	—	November	24
	67	28	December	7
	28	67	Unknown	26
	—	—		—
	95	95		63

The large number of cases that occurred in the year 1901 indicates that some exceptional condition existed that changed an unimportant and infrequent complication into a very important and frequent one.

The occurrence of tetanus as a complication of vaccination has been variously explained as follows:

1. *That it is an accidental secondary infection of the vaccination sore.* Those holding this view think it depends upon conditions ever present and only to be expected, and that its occurrence is deplorable and preventable by the exercise of greater skill and caution in the performance of the operation

and the subsequent treatment of the wound. That such a microörganism as the tetanus bacillus, whose natural habitat is the soil and which occurs widely disseminated in Nature, might occasionally be able accidentally to find its way into vaccination lesions, cannot be gainsaid. It is certainly possible, and may occasionally happen, but there are very cogent reasons opposed to this view, and to content one's self with such a simple explanation may be to fall into egregious error, for if tetanus can thus occur, it should do so in all parts of the world, with more or less regularity.

The various indices to the medical literature are without any references to cases of tetanus following vaccination in the continental countries. A communication from the Imperial Health Office of Berlin informs me that the complication is *unknown in the German Empire.* Letters from the Pasteur Institute at Paris, the Pasteur Institute at Lille, and the French Institute inform me that the complication is *unknown in France.* No cases appear to occur in Italy, Russia, Austria, or other European countries, so that we are engaged in the consideration of a complication that is chiefly American, has become important within a year, cannot be reasonably attributed to geographic, telluric, atmospheric, or social conditions, but must depend upon some mode of operating, some mode of preparing the virus, or some mode of treating the wound in vogue at the present time, but not in the past.

2. *That the occurrence of the complication depends upon a local prevalence of the tetanus bacilli.* The epidemic in Camden has been attributed to germs in the dust of that city, supposed to depend upon a prolonged period of dry weather. This entirely fails to explain the matter, for were this the true solution we should find ordinary traumatic tetanus occurring more frequently than usual. This is, however, not the case, as aside from the vaccination cases there were fewer than the usual number of cases both in Camden and Philadelphia. Further, while it is true that a greater number of cases occurred in Camden than in other places of equal size, the occurrence of tetanus following vaccination has too

wide a geographical distribution to be explained in this way.

TABLE III.

Report of the Deaths from Tetanus in Camden City for the following Years.

	1901	1900	1899	1898	1897	1896	1895	1894	1893	1892	1891	1890
January	....	....	....	....	....	....	....	....	....	....	....	....
February	....	....	....	....	....	....	....	....	....	....	1	....
March	....	....	....	....	....	....	....	....	....	....	....	....
April...........	....	....	....	....	....	....	....	....	....	....	....	....
May	....	....	1	....	1	....	....	....	....	....	....	....
June	....	....	....	....	....	....	1	....	....	....	....	....
July............	1	....	....	....	....	....	....	....	....	....	....	....
August	....	....	....	2	....	....	....	....	....	....	....	....
September	....	....	....	1	....	....	....	1	....	....	....	1
October	....	....	....	....	....	....	....	....	....	...	....	....
November	9	....	....	....	....	....	....	....	....	....	....	....
December.......	1	1	....	....	....	....	....	....	....	....	....	....

Cases are reported from seventeen different States in the United States, from the Dominion of Canada, Porto Rico, the Philippine Islands, Cuba, etc.

That geographical conditions may exert a pronounced influence upon the occurrence of the tetanus, tetanus being known to be particularly prevalent in certain districts. Thus it is said that Cuba and Porto Rico have soils particularly rich in tetanus bacilli, and that Long Island has a similar dangerous soil. That such an influence is important is, however, very doubtful, as from these territories we have very few cases reported. (3 Cuba, 3 Porto Rico, 2 Long Island.)

TABLE IV.

Tetanus Cases — Showing the Geographical Distribution.

	Cases.	Rumors.
Canada.		
Three Rivers, Quebec	1	
St. John, N.B.	1	
Connecticut.		
South Glastonbury	1	
Cuba.		
Havana	3	
England.		
Lancet	1	
Commissioners' Report	1	
Illinois.		
Chicago	0	1
Louisiana.		
New Orleans	1	
Maine.		
Biddeford	1	
Maryland.		
Baltimore	1	
Massachusetts.		
Belmont	1	
Cambridgeport	1	
East Dennis	1	
Boston	1	
Michigan.		
Kalamazoo	1	
Minnesota.		
Minneapolis	1	
Taylors Falls	1	
Missouri.		
St. Louis	0	1
New Jersey.		
Atlantic City	4	2
Bridgeton	1	
Camden	11	

	Cases.	Rumors.
New Jersey.		
Jordantown	0	1
Millville	1	
New York.		
Albany	1	
Long Island	2	
Oswego	1	
Ocean.		
"City of Para"	1	
Ohio.		
Cincinnati	0	1
Cleveland	4	
Pennsylvania.		
Bristol	2	
Easton	1	
Girardville	1	
Lyndell	1	
Millbach	0	1
Philadelphia	11	12
Rosemont	0	1
Philippine Islands	1	
Porto Rico	1	2
Scotland	1	
South Carolina.		
Charleston	1	
Tennessee.		
Paris	1	
Unknown	0	6
Virginia.		
Richmond	1	
Wisconsin.		
Milwaukee	1	
	67	28
	28	67
	95	95

3. *That carelessness in the treatment of the vaccination wound is the source of the difficulty, because it facilitates accidental secondary infection.* While this has a ring of genuineness about it, the argument is extremely unconvincing, if one take into consideration the fact that for one hundred years vaccinations have been performed with a total disregard to cleanliness and asepsis in all parts of the world, on all classes of people, in towns and cities, by careless and careful physicians, upon clean and dirty persons, and that it has been unusual for any dressing to be applied in the past, and that during these many years tetanus has remained almost an unknown complication. We cannot conceive of any difference between the social conditions at the present and those of the past that are not immensely in favor of the present; yet it is at present that we find tetanus.

In our own country greater care is undoubtedly exercised at the present time than heretofore, and in those cases in which, because of ignorance and poverty, the same conditions prevail at the present time that existed a hundred years ago, as for example among the peasantry of Europe, we find tetanus practically unknown.

Vaccination wounds are treated with much greater care at the present time than ever before. It is but now becoming recognized that vaccination is an operation, and that to free it from the common dangers of all operative manipulations, cleanliness and care are essential. We, therefore, find that at present the skin is cleansed and disinfected and the lesion itself protected and subsequently dressed with a care never dreamed of a few years ago; yet when we come to examine the details of those cases which have come within our knowledge, we find that this care of the wound appears to be without influence upon the development of tetanus, for while many cases have occurred among ignorant and filthy children, in an equally great number of cases not only ordinary but extraordinary care seems to have been exercised. Thus one case occurred in the person of an adult sister of a physician in Cleveland, Ohio. Both the patient and the operator were refined and cultured people, were apprehensive of the results,

and exerted unusual and extreme precautions, yet despite all, death from tetanus followed this vaccination. Here let it be said that the virus apparently responsible for so many cases was used.

The densely ignorant and filthy people of the island of Porto Rico, with no knowledge of personal hygiene, living in a place reputed to be extremely dangerous because of tetanus, were vaccinated by the United States authorities after the occupation of that territory, and out of some eight hundred thousand vaccinations three cases of tetanus, two of which are very doubtful, are reported by Dr. George G. Groff.

4. *The use of a shield to protect the vaccination wound has recently become quite common, and has been blamed for the occurrence of tetanus.* It has, however, met with violent and perhaps justifiable condemnation, on the ground that the pressure of its edges and of the adhesive plaster bandages by which it is held in place obstructs the lymphatic circulation and increases the severity of the wound and the danger of infection, also that the shield induces anaerobic conditions. Upon looking over the cases reported, however, we find so few in which shields were used that they seem to have had no influence upon the occurrence of tetanus.

The Relation of Tetanus to the Vaccine Virus.

With the evolution of vaccination as a prophylactic measure, certain changes in technique have been gradually introduced. Thus bovine virus has now displaced the arm to arm and human scab vaccinations previously employed, and within the last quarter-century the use of the human virus has been almost entirely given up. Can it be that the danger of tetanus has something to do with the employment of bovine virus? Only a few of the early cases drawn from the literature followed the use of human virus. This seems possible when we consider that it is only in the last half century that any cases have occurred, but can scarcely be, inasmuch as bovine virus is the only form employed in Belgium, Germany, France, and other European countries, yet we find

no tetanus there, though it is common in our own country at the present time. While it is suggestive that the first tetanus cases reported in the literature make their appearance about the time bovine virus came into general use, it is not true that the number of cases increased in proportion to the popularity of the bovine virus; for from 1854 when the first case makes its appearance in the literature until 1901, only isolated cases usually at intervals of years are reported, a sudden extraordinary increase being observed in 1901.

The next step in the evolution of vaccination was the improvement in the quality of the virus, suggested by Copeman in 1891, by which glycerine was permitted to act for some time upon the virus, for the purpose of destroying microörganisms which it contained. By the use of the glycerine, a bacteria-free virus can be secured. This improved virus made slow but steady progress, until at the present time it has attained to the greatest popularity and bids fair to replace all other forms. It is used almost exclusively on the continent of Europe and has made very large inroads into this country.

The glycerinized virus is so new, having been in use but ten years and in common use little more than five years, that we are scarcely able to express a positive opinion regarding its advantages and disadvantages. The advantage of having the contaminating bacteria destroyed by the glycerine is indisputable; the disadvantage of occasionally having the glycerine act so long that the specific germs of vaccinia are killed and the virus made inert, is also indisputable. That at times when the demands upon the manufacturer are exceptionally great, the virus mixed with the glycerine might be placed upon the market before the necessary time had elapsed for the contained bacteria to be killed, is a somewhat grave danger. These possibilities have somewhat lessened the confidence with which the preparation is received and used by many practitioners.

When we come to investigate the cases of tetanus concerning which information is at hand, we are somewhat startled to find that a large number of them have succeeded the employment of this supposedly best and most refined preparation. This point will be discussed below.

TABLE V.

Showing Relation of Tetanus to Make of Virus.

	DRY.	GLYCERINIZED. Points.	GLYCERINIZED. Tubes.	
E.	3	10	17 =	30
A.	2		=	2
D.	0		2 =	2
W.	0	1	=	1
S.	0		3 =	3
I.	1		=	1
G.	1		=	1
	7	11	22	40 cases.

Relationship to Particular Brands of Virus.

When an attempt is made to determine whether any particular brand or make of virus yields an unusually large number of tetanus cases, we are confronted by a still more suggestive fact; that is, that while an occasional case of tetanus has succeeded the use of the virus of nearly all of the large manufacturers, the great majority of the cases have succeeded the use of a particular brand of virus.

The brands of virus to be considered are called E., D., A., S., W., I., St., and Wd. Those called E., A., and D. are the products of the largest manufacturers, and it is not improbable that they do about an equal amount of business. It is probably safe to say that these vaccines are all made under careful conditions, with the exercise of as great an amount of skill as can be exerted with our present knowledge. No care or expense is spared to have these products perfect. We find, however, a remarkable discrepancy in the results following their employment — a discrepancy that leads me to conclude that tetanus bacilli may be contained in the virus and distributed with it.

The occurrence of thirty cases of tetanus after the employment of virus E. indicates that in it tetanus germs were present in larger proportions than in any other, though the occurrence of only thirty cases following the use of millions of

doses indicates that the number of tetanus organisms present was small.

The Occurrence of the Cases in Groups.

In this connection must be pointed out as interesting and significant the groups of cases that have occurred from time to time. Thus in Cleveland, O., during 1901, we note the occurrence of four cases. In Camden, N.J., during October, November, and December, 1901, the occurrence of eleven cases. At Atlantic City, about the same time, five cases, and in Philadelphia and vicinity nearly at the same time about twenty-five cases. If we analyze these cases we find one vaccine (virus E.) chiefly if not exclusively implicated. The most instructive group of cases that I have been able to study occurred in the Philadelphia Hospital. In this institution with nearly four thousand five hundred inmates, there was a threatened epidemic of small-pox depending upon the admission of a case of small-pox from the street. After one or two cases had developed within the institution, it was decided to vaccinate every inmate, and the resident physicians went systematically through the institution vaccinating sick and well alike. The institution was thus vaccinated with the exception of the Men's Insane Department, the greater number of whose inmates were obliged to wait a few days until a new consignment of virus arrived. With this new consignment (virus E.) they were then all vaccinated. Upon looking up the statistics of the hospital, we find that in the insane departments, male and female, no case of spontaneous traumatic tetanus had developed within twelve years. It is, in fact, not known that there ever has been a case of traumatic tetanus developed within the walls of the institution, but this cannot be determined, as the records prior to twelve years ago were destroyed by fire. Succeeding the vaccination, however, a group of tetanus cases, confined exclusively to the Men's Insane Department, occurred. Here five typical cases with trismus and opisthotonos and every marked symptom of the disease occurred, all being followed by death, four from trismus itself, and one from pneumonia, occurring im-

mediately after a cessation of the spasms. The occurrence of this outbreak occasioned much alarm, so that every suspicious vaccination wound observed was thoroughly excised and treated antiseptically. After this excision of the wounds, *eleven additional cases developed* trismus and muscular rigidity, though after the administration of enormous doses of antitoxin they all recovered. In going carefully over the details of these cases, I find that with one very doubtful exception, every patient that developed tetanus was vaccinated with the same virus which had caused tetanus at Cleveland, Camden, Atlantic City, Philadelphia, and elsewhere (virus E.).

Should it be suggested that the occurrence of groups of tetanus cases depends upon the popularity of the virus in certain districts, as Philadelphia, Camden, Atlantic City, where it was almost exclusively used, and that in these districts some telluric, atmospheric, or other condition prevailed, causing them to be more predisposed to tetanus than the country in general, the idea should be at once dispelled by a few moments' consideration of the statistics presented. Thus if we deduct from the total thirty cases attributable to virus E., the six positive cases occurring in Camden, the seven positive cases occurring in Philadelphia, and the three positive cases occurring in Cleveland, there remain against this virus a total of fourteen cases, which is greater than the sum total of the cases referable to all the other viruses produced in the country, thus showing that even where the cases are scattered, and not in groups, this virus E. has four times as many cases to its credit as any other one virus and many more than all the other viruses put together.

Thus convinced that the bacilli which cause tetanus subsequent to vaccination are in the virus, we are obliged to prove our position, which we do by a statistical study of the material collected. (Refer to Table IV.)

Forty cases are presented with exact information concerning the make and form of virus employed. Of these forty cases *thirty* follow the employment of virus E., *ten* follow the use of all other forms combined, and no other single virus

has a higher number than *three*. This seems to show quite convincingly that there is something about virus E. that is different from the others. We have in addition to these forty cases with complete details, eight cases in which it is known positively that one or the other of two viruses was used, but in which it cannot now be certainly determined which of the two it was.

TABLE VI.

Cases of Disputed Virus where Names are Given.

Cases, No.[1]	E.	A.	D.	W.	Wd.	St.
25	1	. .	. .	1	. .	. .
29	1	. .	1	. .	. .	. .
45	1	. .	. .	. .	. .	1
56	1	1	. .	. .	. .	. .
71	1	1	. .	. .	. .	. .
72	1	1	. .	. .	. .	. .
73	1	1	. .	. .	. .	. .
91	. .	. .	1	. .	1	. .
	7	4	2	1	1	1

We find that in the cases of this group vaccine E. figures in seven. If we assume, which it would be fairly justifiable to do, that all of these cases belong to E., then the disproportion is changed from

E., 30: all others 10; to
E., 37: all others 11;

thus making matters much worse. If, however, we proceed on the reverse order and admit that E. may be erroneously charged with these cases, we find the proportion

E., 30: all others 18,

so that matters are but little improved for virus E.

[1] The numbers refer to my list of cases, which cannot be published in detail, because much that it contains is confidential.

TABLE VII.

Table of Comparisons.

Viruses.	Known.	Disputed.
E.	30	7
A.	2	4
S.	3	0
D.	2	2
W.	1	1
Wd.	0	1
St.	0	1
G.	1	0
I.	1	0

Dry and Glycerinized Viruses.

The respective influences of dry and glycerinized virus upon the causation of tetanus is shown by reference to Table V., where we see that seven cases followed the use of dry points, eleven the use of glycerinized points, and twenty-two the glycerinized tube virus. This proportion, seven to thirty-three, appears to be convincingly in favor of the dry virus, but we find an error resulting from the use of virus E. All three forms of virus E. have been followed by tetanus in the respective proportions of dry points three, glycerinized points ten, and glycerinized tube virus seventeen. If we entirely omit virus E. from the consideration, we find the totals of all other viruses to be:

Dry points,		4
Glycerinized points,	1	$\Big\} = 6$
Glycerinized tubes,	5	

The proportion of four dry to six glycerinized being about correct when we remember that the glycerinized is the popular virus at the present time.

This seems to show pretty well that there is no disproportion in favor of dry over glycerinized virus as regards the occurrence of tetanus.

The Source of the Tetanus Bacilli in Virus.

When we consider the distribution of the tetanus bacillus in nature, we find it in the soil, chiefly where it has been well fertilized. The tetanus bacillus is, no doubt, frequently swallowed by herbivorous animals in browsing upon the surface of the ground. The anaerobic conditions for its growth being excellent, we find that the intestines of herbivorous animals commonly contain large numbers of the bacilli, and that with the intestinal evacuations, the bacteria are deposited again upon the soil in increased numbers. The first source of danger, therefore, by which vaccine virus can be contaminated is the manure of the calf, the next source the dusts arising from the dry manure and the soil, both of which may be brought into the stables with the hay, or upon the animals.

When large hay fed animals are employed for the manufacture of virus, the manure is certainly a source of danger, but in stables where sucking calves are used for the purpose, fewer tetanus bacilli are present in the excrement. However, Huddleson found them present in the feces of eight per cent of the small calves used in the laboratory of the Health Department of New York.

It is evident that from these sources opportunities occur for the entrance of tetanus germs into the vaccine virus, but as a matter of fact no one has yet succeeded in finding them in the virus. This indicates that their number must be so small that in the distribution of the virus in tubes and on points, hundreds of thousands of tubes escape where one tube is contaminated. From these data we have been forced to conclude that it is unusual for many tetanus germs to be implanted at the time of vaccination, but that such implantations can and do occur.

The Incubation Period.

The period of incubation of ordinary traumatic tetanus varies from a few hours to several weeks (see Plate XL., Chart A), according to statistics derived from the study of a large number of cases.

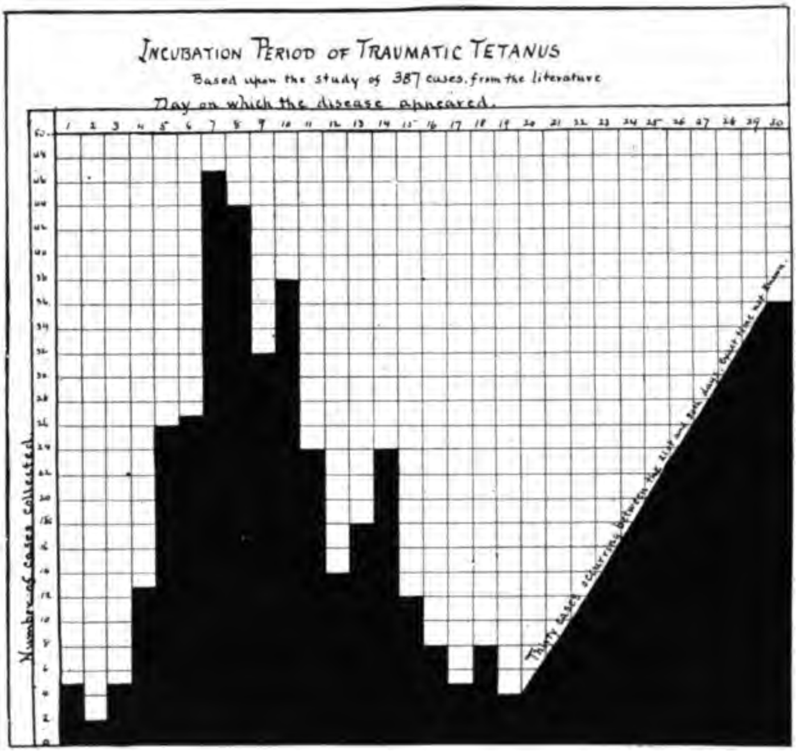


CHART A.

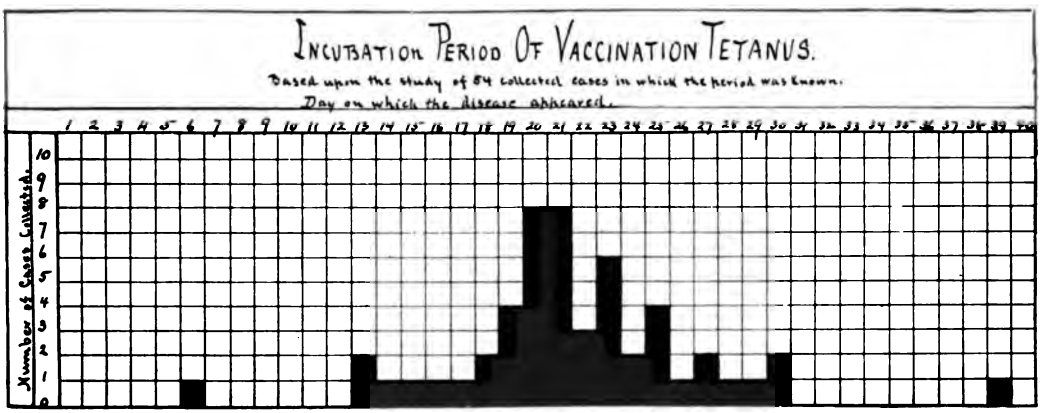


CHART B.

TABLE VIII.

Tetanus Cases — Showing Incubation Period.

Shortest period	6	days.
Longest period	39	"
Average	22	"
1. For Points — Shortest period	30	"
Longest period	16	"
Average	23	"
2. For Glyc. Virus — Shortest period,	6	"
Longest period	39	"
Average	22	"

The usual incubation period varies from seven to nine days. In the vaccination cases, that is, in cases of tetanus following vaccination, we find the shortest period of incubation to be six days, the longest thirty-nine, the average twenty-two days (Plate XL., Chart B). It is thus seen that tetanus following vaccination appears on the average about two weeks later than ordinary traumatic tetanus. This is the sole weakness in the argument, and I think if this error could be eliminated it would at once be conceded that the remaining facts are indisputable. The prolonged incubation period is usually interpreted to signify that the infecting organism entered the vaccination wound at a time when it was open, exposed, and subject to secondary infection from the air, water, clothing, etc., with which it comes in contact. In truth, however, how rarely do superficial ulcerations become infected with tetanus! Did any one ever hear of a sudden paroxysmal outbreak of sixty-one cases of tetanus in one year following the infection of leg-ulcers, furuncles, mosquito bites, etc., even when cleanliness was not observed?

I think after the statistical demonstration given, that this secondary infection theory must be looked upon as a misinterpretation. The probability is that the tetanus bacillus is ingrafted into the skin at the time of vaccination, but fails to find suitable conditions for its growth until after the development of the vaccine lesion paves the way by the local

destruction of tissue. This makes us add to the usual incubation an additional period (a couple of weeks) during which the wound has been prepared for the implanted organisms, thus bringing the entire period to the length usually observed in the vaccination cases. An examination of Table VIII. shows that no difference exists between dry vaccine on points and glycerinized virus regarding the length of the incubation period.

In nearly every case of which I have succeeded in securing details, I learned that the vaccination "took well." This may not be without significance, though it may be interpreted in several ways. It may mean that those vaccinations in which severe local lesions occur are those in which secondary infection has the best chance of occurrence. If so, it means that such lesions are dangerous and that mild viruses should be used, the multiple insertion of a mild virus being preferable to a single insertion of an active virus. It may also mean that the lesion was caused by an impure virus, in which tetanus as well as other organisms might be contained. Lastly, and I think truly, it may mean that it is only when such local lesions occur that the implanted tetanus bacilli can find conditions suitable for their development.

Reference is again to be made to the chronological development of the cases, by which it will be observed that the majority of cases occur at a time when, because of agitation concerning small-pox, the demands for virus are great. This being the case, it is not impossible that vaccine viruses are sometimes marketed prior to the time when the action of the glycerine has extinguished the life of the bacteria in the virus.

Conclusions.

From the foregoing the following conclusions seem justifiable:

1. Tetanus is not a frequent complication of vaccination, a total of ninety-five cases having been collected.

2. The number of cases recently observed is out of all proportion to what has been observed heretofore.

3. The cases are chiefly American and occur scattered throughout the eastern United States and Canada.

4. They have nothing to do with atmospheric, telluric, or seasonal conditions.

5. They occur in small numbers after the use of various viruses.

6. An overwhelming proportion has occurred after the use of a particular virus.

7. The tetanus organism may be present in the virus in small numbers, being derived from the manure and hay.

8. Occasionally the number of bacilli becomes greater than usual through carelessness or accident.

9. The future avoidance of the complication is to be sought for in greater care in the preparation of the vaccine virus.

[Since writing this paper, the final proof of its correctness has been given by the discovery of tetanus bacilli in some of the same vaccine virus used at the time the chief outbreak occurred in 1901. This discovery was made by Dr. R. W. Wilson, and communicated to the Philadelphia County Medical Society on April 23, 1902.]

ARBOREAL TRAITS IN THE HUMAN FOOT.

E. H. Bradford, M.D.

It has been claimed that arboreal traits are to be seen in the grasp of the new-born baby's hand, which, as is well known, is sufficiently strong to hold the infant's whole suspended weight.

If arboreal traits are found in the hands, it is reasonable to infer that they exist also in the foot. It is, therefore, of scientific interest to determine whether there is any evidence of arboreal ancestry to be found in the movements of the human infant's foot.

By arboreal traits in the foot are meant any survival of grasping power similar to that seen in the foot of the quadrumana. In order to determine this, an examination was made of the feet of a number of new-born infants, from a few hours to a few days old.

The foot of an infant a few hours old is usually held in a position of slight calcaneous. Although a position of slight supination is not uncommon, it is not seen as frequently as the calcaneous position, *i.e.*, with the sole facing directly downward and the front of the foot drawn somewhat upwards.

The first movements of a new-born infant's foot upon being irritated are those of the toes. These are raised in the dorsal direction, but with an irregularity of movement in the different toes, the first and fifth toes moving independently of the others and of each other; the middle toes moving more nearly together. This dorsal extension is frequently followed by plantar flexion, also irregularly, the great toe being raised while the others are drawn toward the sole. The foot is also spread, the first and fifth toes being drawn to the inner and outer sides of the foot, the first being drawn to the inner side to an angle of twenty degrees from the middle line; the amount the fifth toe is drawn to the outer is less, though a divergence of ten degrees from the middle line is not uncommon.

If slight pressure is made on the central and forward portions of the foot, the toes will be curled toward the sole, the first and fifth toes being drawn toward the middle line of the foot as if to grasp anything placed under the sole. The side movements of the great toe are more free than those of the others.

If anything is placed between the first and second toes, there is a slight grasp, and the same is true if the toes are curled around a small rod. The various motions of the toes resemble those of the fingers, except that they are much more limited in extent, and the power of opposition of the great toe to the others is much less than that of the thumb to the fingers. The thumb action of the great toe is rudimentary. The movements of the infant's foot are, therefore, not unlike those of the hand, if instead of the thumb a finger were added with as much mobility but greater strength than is found in the little finger.

The toes more commonly move toward the dorsum than toward the sole, perhaps a survival of the quadrupedal attitude of the foot; but the powerful plantar flexion of the toes indicates that the claw-like action of the toes also survives.

The difference in length of the phalanges of the foot from those found in the hand limits the grasp; and it is clear that while the foot is perfectly adapted to the chief work for which it is designed, and can be developed to a moderate degree as an implement of grasp (as is seen in many barefooted races and in the armless individuals where the foot has been trained), there is no evidence of the possibility of developing a condition resembling the foot of the quadrumana, which is as strong in its grasp as the hand, or in the ourang even stronger.

The human foot differs from that of other animals chiefly in the relation of the heel to the rest of the foot. In other animals than man, the os calcis acts as a short lever behind the ankle to which a strong muscle is attached, rather than a weight-bearing bone; that is to say, the os calcis either does not touch the ground in locomotion, or touches in such a way that the greater part of the weight is borne on the front

of the foot. That is necessarily the case in quadrupeds, and is seen in creeping infants. As the infant becomes a biped, the end of the os calcis is used to sustain the greater part of his weight when standing. The front of the foot is used to aid in standing, and for downward and backward pressure on the ground in walking and running. An arch of the foot is formed and the weight is borne upon the two ends of the arch with, in addition, a greater part of the outer edge of the foot.

In all other animals the weight falls upon the phalanges alone or the metatarsal bones with the phalanges. Even in primates where the os calcis touches the ground, an arch of the foot does not exist, some weight falling upon the scaphoid and little or none on the end of the os calcis.

In walking, the end of the os calcis in man serves not only to sustain the weight, but in the erect gait furnishes a pressure point on the ground; the thigh muscles act in pulling the individual forward to this point in one movement of the stride, followed by the action of the front of the foot in the completion of the step, pushing the individual forward.[1]

The os calcis in man as in other animals acts as a point of attachment for the calf muscles, permitting the use of the whole of the foot in front of the ankle to serve as a lever for downward and backward pressure in walking and running. In the mid tarsal articulation, the up and down motion seen in the quadrumana and many other mammals is limited in man, and the absence of this motion gives a greater firmness to the foot in front of the ankle, and adds materially to the power of the foot as a lever implement of locomotion. It is manifest that the longer and firmer the foot in front of the ankle, the greater is the power of this lever action necessary for rapid locomotion. In the quadrumana the mid tarsal articulation is more free than it is in man, increasing the arboreal utility of the foot while decreasing the power of rapid locomotion. A certain amount of side motion of the medio tarsal articulation remains in the human foot, and aids materially in enabling the individual to maintain the balance necessary

[1] New York Medical Journal, January, 1900, page 109.

for rough walking and climbing. Steadiness is also given by the spreading of the metatarsals, especially the first and the fifth which broaden the base of support. Strength of gait is increased also by the strong first metatarsal and its corresponding digits, characteristic of the human foot. The other metatarsal and phalangeal bones aid in the locomotion, but also largely in giving steadiness and a pliability of the foot for rough travelling. The claw-like action of the toes and the flexibility of the first and fifth metatarsals are also of great aid in rough walking and climbing. In short, the foot is admirably adapted for furnishing firm and secure support in the erect attitude, enabling the individual to have free use of the arms and hands. It is to this firm footing that man owes the possibility of the development of deftness in his hands and of effectiveness in offence. His nimbleness and strength of foot enable him to pursue successfully and to escape from other animals more powerful than himself.

Man can creep, walk, climb, run, and swim, but he is entirely unadapted to any active tree life. It is due to his superior foot that primitive man was so successful in the combat with other animals, and, as has been shown, drove the more powerful ape from the continent of Europe to the impenetrable forests of Central Africa.[1]

The grasp of the hand seen in the infant is not necessarily to be regarded as an arboreal trait, for if the foot is not adapted to tree life, it is natural to explain, if possible, the characteristic reflex action in the hand in some other way. It has been suggested that the grasp is necessitated by the need of the child clinging to the mother, as is seen in the grasp of the infant ape to the hair of the mother, who is unable to hold her offspring in her arboreal flight, where she needs both hands and feet.[2]

It seems probable that the grasp of the hand is simply a natural reflex in an infant, a life-preserving instinct like the swimming instinct seen in infants; the instinct to hold firmly what it seizes, grasping the mother's clothing, holding firmly

[1] Sir Harry H. Johnson. Pigmies. McClure, February, 1902, page 349.

[2] This is perhaps true in a measure of the Abyssinian infant. New Trails in Abyssinia Country. Century Magazine, April, 1902, page 889.

food given later. It is as necessary to the hunter in the grasp of a weapon of attack or defence as is the quickness or sureness of foot, and the human infant is given this power so needful in after life to a larger degree.

Simian resemblances which have been mentioned in the human foot are as follows: [1]

1st. The position of supination frequently assumed in the infant foot.

2d. The abduction and adduction power and position of the great toe.

3d. The amount of dorsal extension of the foot.

In addition to this the comparative shortness of the lower extremities in infancy has been mentioned as a Simian resemblance.

The position of supination of the foot is frequently assumed by infants, more by older infants than by the newly born. It is entirely unaccompanied by any power of grasping pressure of both feet necessary in climbing. If an infant's feet are placed with the soles opposing and an adult's finger, hand, or wrist is placed between them, there is no pressure on the fingers by the infant's foot. If there were a vestige of tree climbing ability to be seen in the foot, such pressure would be expected if the same is found in the clasp of the hands, but on the contrary no power is found in the foot in this direction.

It is rational, therefore, to regard this occasional position of supination of the foot, not as an arboreal trait, but as the result of the cramped position of uterine life. The power of supination remains and is important in the activity necessary for man as is the power of pronation.

The abducting power of the great toe is of importance in furnishing a broad base for the foot needed in standing; it aids greatly, therefore, in making man an erect animal. This is also found for the same purpose in the fifth toe, though to a less degree. This mobility is seen in all barefooted races and gives steadiness to the foot.

---

[1] Lazarus. Morphologie des Fussskelets. Morpholog. Jahrbuch, B. 24, H. 1, p. 1–166.

Where the great toe is separated strongly from the others, it is manifest that unless it can be drawn towards the median line of the foot, running would be interfered with as the projecting great toe would strike the other leg in rapid motion. The power of adduction of the great toe, therefore, must equal the power of abduction. This gives a slight power of grasp which is utilized by the Japanese and Filipinos and others. This may be developed to a high degree, which becomes of great usefulness to the armless.[1] As has already been said, its chief use is to give great firmness and strength to the human foot, which is characterized by the strength of the first metatarsal and its phalanges. To this is added some flexibility, but if as much flexibility were given as is given to the primates in the grasping ability of the thumb and foot man would be slower of foot.

The amount of dorsal extensibility of the toes and of the foot reverts more to a quadrupedal than to a quadrumanal state — a condition strongly indicated in the embryo, in the primate pads,[2] and which can be classed as one of the vestiges of a primary condition which though early was not ape-like.

The shortness of the lower extremity is hardly Simian; like the large size of the fetal head, it is to be explained as a phase of growth, one portion being developed at a different period of growth from another. The new-born infant has little need for its lower extremities.

Comparative disuse of the thumb of the hand as a grasp, seen in the hands of monkeys, is also seen in babies when grasping anything to which they are not trained, and this has been claimed as a Simian vestige. In the human infant this can be explained as a power not yet trained, but easily acquired; in an ape, by the fact that in the foot in the ourang and other climbing apes, the thumb of the foot is more of a thumb than the thumb of the hands, and is used constantly in grasping, and is needed in arboreal life. In human infants the foot is developed as a means of support upon the ground, and the hand for a strong grasp.

---

[1] Cast of the foot of an armless man in the Ethnological Museum in the Jardin des Plantes.

[2] Demonstrated to me by the kindness of Prof. C. S. Minot.

Other Simian vestiges which have been mentioned are bow-legs seen in the new-born infant, due more probably to the position necessitated by uterine life.[1]

To describe the grasp power of the hands and the clenched position of the hand usually seen in infants, or the climbing instinct of children, as Simian vestiges, seems lacking in scientific accuracy, as both are needed in human as much as in ape life.

Man shows in infancy vestiges of a primitive condition, which was quadrupedal, but the foot shows fewer Simian than quadrupedal resemblances. The human foot resembles the foot of a bear or opossum as much or more than it does that of a chimpanzee.

To describe the foot as a "degenerated hand," as has been recently done, is hardly correct. It can be regarded as an admirable implement for one of the chief occupations of the primitive man, viz., hunting, showing the same remarkable adaptability of the original design for the purpose needed in man, as is to be observed universally in the animal kingdom, notably in the bat and in the seal.

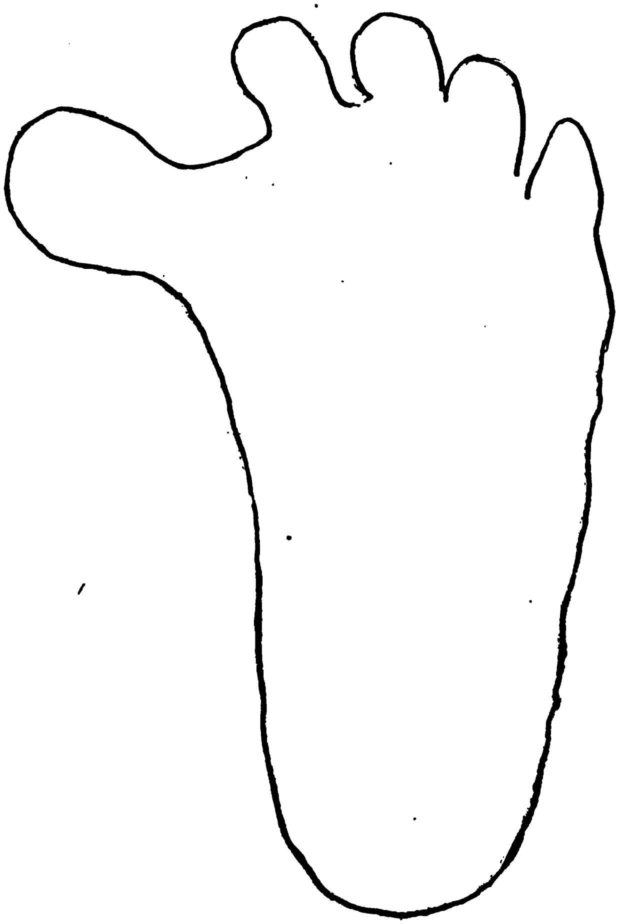
The persistence of the type of the human foot is a fact of interest. The skeleton of the foot of a pigmy of Central Africa (South Kensington Museum) shows a distinctly human foot. This is also true of the bones of the prehistoric man found in the caves of Dordona (*v.* Ethnological Museum, Jardin des Plantes). There is the same limitation to the divergence of the great toe and the limitation of the rotary play at the first metatarso-tarsal articulation and at the mid-tarsal articulation. There is the same strength of the first metatarsal and its phalanges as compared to that of the other long bones of the foot. This is also seen in the prehistoric footprint from Nicaragua mud to be seen in the Cambridge Peabody Museum.

There is but little variation to be seen in the human foot in the various human races. An unusual divergence of the great toe has been claimed to exist among the Annamites.

---

1 Pearson's Magazine, April, 1902, p. 393.

It is also seen in some Filipinos, where it is probably developed by occupation. In no case is it greater than seen in the ordinary infant. The type of the human foot may be regarded as well fixed and with little tendency to revert.



TRACING OF FOOT OF A PHILIPPINE BAMBOO CUTTER.

Congenital deformities frequently reveal a reversion to a primary condition. The webbed fingers and toes occasionally seen may be regarded as an attempt at reversion (like a hare lip and branchial fissure). It has been claimed that

club foot (talipes equino varus) is a form of reversion to an arboreal type. A careful study of the anatomy of this deformity and a comparison of this with that of the foot of a chimpanzee does not support this idea. Club-foot, congenital or acquired, is a dislocation at the medio-tarsal articulation, and in the congenital form it is to be classed with other congenital dislocations of joints of uterine origin, as is the case with the congenital dislocation of the hip, which is in no sense a reversion.

That man presents vestiges of a primitive state from which the ape descendants with their arboreal life were also evolved is undoubted. He probably presents also evidences of Saurian ancestry. That man himself ever enjoyed arboreal life is not clear, and needs stronger proof than is to be found in the human foot.

The unarmed defenceless condition of man made him gregarious, in self protection. This aided in the development of his intelligence, as it is only by the display of mental aptitude that he has been able to outwit his enemies and withstand the rigors of existence. The long infancy of his offspring developed a sense of protection, social instinct, and the higher virtues; but it is his strength and sureness of foot which enable him to maintain and defend the civilization he has acquired.

The grasp of the new-born baby's hand gives promise of the power of vigorous offence with any weapon within his clutch; the reflex movements of the foot reveal the capabilities of a future Nimrod, " a mighty hunter before the Lord," which centuries of civilization have not destroyed.

DESCRIPTION OF PLATE XLI.

FIG. 1. — LEVER ACTION IN FOOT. Above is human, below is ape. The dotted line indicates the mid-tarsal articulation.

FIG. 2. — Diagram indicating movements seen in a new-born human foot.

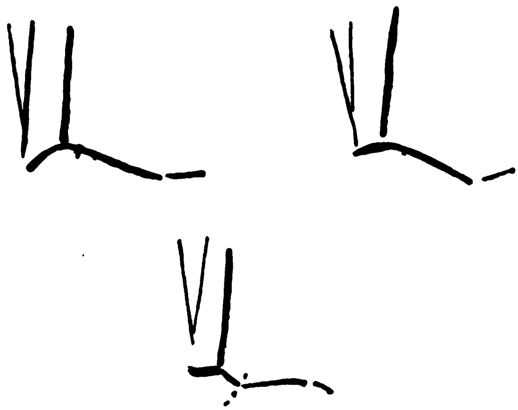


FIG. 1.

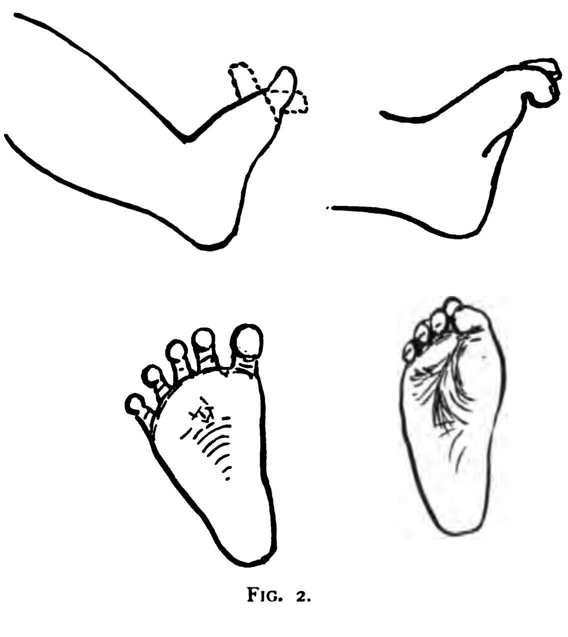


FIG. 2.